



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Goddard, et al.
Appl. No. : 10/006,867
Filed : December 6, 2001
For : SECRETED AND
TRANSMEMBRANE
POLYPEPTIDES AND NUCLEIC
ACIDS ENCODING THE SAME
Examiner : Larry R. Helms
Group Art Unit : 1642

DECLARATION UNDER 37 CFR §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I declare and state as follows:

1. I am a joint inventor of the invention claimed in the above-captioned patent application.
2. During the time period in which I participated in the events and activities described herein, I was employed by Genentech, Inc., the assignee of the above-captioned application.
3. All of the events and activities described herein were performed by me personally, by others at my direction, or by the other joint inventors, as part of our duties as employees of Genentech, Inc.
4. The invention claimed in the above-captioned patent application was conceived prior to May 1999 and diligently reduced to practice thereafter in the U.S. as described below.
5. Prior to May 1999, I and/or my joint inventors conceived of the invention claimed in the above-captioned patent application. Prior to May 1999, the idea of investigating several newly discovered DNA sequences for their relevance, including developing primers and cloning the DNA sequences of interest from normal and tumor tissues, was conceived. The attached sequence printout (Exhibit A), dated prior to May 1999, shows the complete sequence of the nucleic acid having the sequence of SEQ ID NO:1, as well as the complete sequence of the amino acid of SEQ ID NO:2. Thus, conception of the invention claimed in the above-captioned patent application occurred prior to May 1999.

Appl. No. : 10/006,867
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6. The date deleted from Exhibit A is prior to May 1999. This date was redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, November 15, 2004.

7. After these initial experiments, I and/or my joint inventor(s), continued to produce primers, clone and sequence other DNA sequences. We then began to identify the expression levels of the cloned sequences, and created constructs for expression of the encoded proteins. The enclosed printed copy of an electronic file (Exhibit B) shows that PCR primers for numerous sequences which had been previously identified were designed on March 6, 2000 (as evidenced by the file name, "oli.out3_6_00"), including the primers for DNA 26843, the DNA which is relevant to the presently claimed invention (see top of page 2 of Exhibit B). This data shows diligence in reducing to practice following conception of the invention. Thereafter, the primers were tested for expression in various normal and tumor tissues on June 13, 2000 (Exhibit C). This page shows a series of gels in which the primers were used to determine tissue and tumor expression levels for the various DNA sequences. The levels are shown using a ++, +, +- and - to indicate the intensity of the specific signal which was detected. The data for DNA 26843 is shown in row 13. This data shows that the DNA is more highly expressed in normal lung tissue than in living tumor, and in rectal tumor as compared to normal rectal tissue. Actual reduction to practice therefore occurred by at least June 13, 2000. Thus, we conceived of the present invention prior to May 1999 and were diligent in reducing the invention to practice by at least June 13, 2000.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: *Audrey Goddard*
Audrey Goddard

Date: 11/29/04

By: Paul J. Godowski

Date: _____

By: J. Christopher Grimaldi

Date: _____

By: Austin L. Gurney

Date: _____

By: William I. Wood

Date: _____

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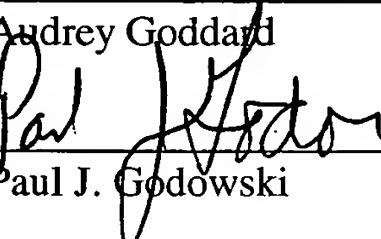
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By: _____

Audrey Goddard

By: 

Paul J. Godowski

By: _____

J. Christopher Grimaldi

By: _____

Austin L. Gurney

By: _____

William I. Wood

Date: _____

Date: 11/24/04

Date: _____

Date: _____

Date: _____

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By: _____
Audrey Goddard

Date: _____

By: _____

Date: _____

By: _____
J. Christopher Grimaldi

Date: 12/2/2004

By: _____
Austin L. Gurney

Date: _____

By: _____
William I. Wood

Date: _____

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By: _____
Audrey Goddard

Date: _____

By: _____
Paul J. Godowski

Date: _____

By: _____
J. Christopher Grimaldi

Date: _____

By: _____
Austin L. Gurney

Date: 11/30/01

By: _____
William I. Wood

Date: _____

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By: _____
Audrey Goddard

Date: _____

By: _____
Paul J. Godowski

Date: _____

By: _____
J. Christopher Grimaldi

Date: _____

By: _____
Austin L. Gurney

Date: _____

By: William I. Wood
William I. Wood

Date: 11/23/04

>>Monday, November 15, 2004
>>DNA26843 [Full]
>>362 Sites [All] Sites

1 TCCAGGTCC AACTGGACCT CGGTTCTATC GATTCGAATT CGGCCCACT CCTAGAGTC TCTGACCTC GACCCACGGC
AGGTCAGG TTGACCTGGA GCCAAGATAG CTAAGCTTAA CCCGGCTAGG AGATCTCTAG GGAGCTGGAG CTGGCTTCCCAGA

cac8I

mwoI

hinP1

haelli/pal-

xhal/cfoI

naiV

eae-

ainPI

nar-

cfr-

ahaII/cfoI

kaiI

mwoI

haerII

hinII/acyl cac8I bfaI

haeII

fnc43I/bsoFI

heeI

aciI

afeI/eco47I.I

banI mspA.I/mspBII

ahaiI/bsaHI [x..xhaI-]

bspMI

hpyC4V

bsgI

ahtII

mspA.I/mspBII

haeII

tagtggct

ggtaaggatt

tacaaaagggt

gcaggatga

agactaacat

tttgtgaagt

tctgtatgtt

aaacacitca

acatgtttgc

f

s

p3

ps

rraI

haelli/pal-

maeI

ainPI

haelli/cfoI

haerII

mwoI

haerII

mbolI

bpuAI

bbsI

hpy-88I

hpyC4V

hpyM1

eco57I

f

s

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xbaI

haelli/pal-

maeI

ainPI

haelli/cfoI

haerII

mwoI

haerII

mbolI

bpuAI

bbsI

hpy-88I

hpyC4V

hpyM1

eco57I

f

s

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f

f

xbaI

haelli/pal-

maeI

ainPI

haelli/cfoI

haerII

mwoI

haerII

mbolI

bpuAI

bbsI

hpy-88I

hpyC4V

hpyM1

eco57I

f

s

f

f

f

f

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f

xbaI

haelli/pal-

maeI

ainPI

haelli/cfoI

haerII

mwoI

haerII

mbolI

bpuAI

bbsI

hpy-88I

hpyC4V

hpyM1

eco57I

f

s

f

f

f

f

f

f

f

f

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f

f

f

f

f

xbaI

haelli/pal-

maeI

ainPI

haelli/cfoI

haerII

mwoI

haerII

mbolI

501	TGGTACTGG AATACTGAGT TGTCTTGGAC TTTCTATTGT GGCAAACTTC CAGAAACCAA CCCCTTTTCG TGCAACATGTA AGTGGAGCTG TGCTTAACCTT AACATGAACC TTAGACTCA ACAAAATCCTG AAAGATAACA CGGAAAAACG ACGTGTACAT TCACCTCGAC AGGAATGGAA	97	V L G I L S C L G L S I V A N F Q K T T L F A A R V S G A V L T F	hpy108III	bbvI	aflIII	aluI	Fnu4HI/bsoFI	hsaI	hsaPI	hpyCH4V	pcII	msII	nlalII
502	GGTGTGTTCA GACCATCCTT TCCTTACCAA TCCATGGCC AACAAAGCTT TCTGGATCAG ACTGTGTTG ACCATACCC AGTAAACATT ACAAACAGT CTGGTAGGAA AGGATGGTT ACGTCGGTT TTAGGTACCG TTGTTTCAG AGACCTAGTC TGACAAACAC	98	hpy108I	bstF5I	hsaI	hsaPI	hsaII	hsaI	hsaII	hsaIII	hsaIV	hsaV	hsaVI	hsaVII
503	GGTGTGTTCA GACCATCCTT TCCTTACCAA TCCATGGCC AACAAAGCTT TCTGGATCAG ACTGTGTTG ACCATACCC AGTAAACATT ACAAACAGT CTGGTAGGAA AGGATGGTT ACGTCGGTT TTAGGTACCG TTGTTTCAG AGACCTAGTC TGACAAACAC	99	hpy1286	hsaI	hsaII	hsaIII	hsaIV	hsaV	hsaVI	hsaVII	hsaVIII	hsaIX	hsaX	hsaXI
504	GGTGTGTTCA GACCATCCTT TCCTTACCAA TCCATGGCC AACAAAGCTT TCTGGATCAG ACTGTGTTG ACCATACCC AGTAAACATT ACAAACAGT CTGGTAGGAA AGGATGGTT ACGTCGGTT TTAGGTACCG TTGTTTCAG AGACCTAGTC TGACAAACAC	100	hpy1287	hsaI	hsaII	hsaIII	hsaIV	hsaV	hsaVI	hsaVII	hsaVIII	hsaIX	hsaX	hsaXI

sphi
 hpyCH4Y r.sphiI
 hgiAI/asphi
 bsp-286 nsphi
 bsi3KAI cac8I
 bny- ddel
 apalI/sncI nlaIII
 alw44I/snoI
 hpyCH4V
 bst4CI/hpyCH4III
 hpyCH4V
 tsp509I
 bsaXI
 nlaI
 70: GATGATCTGGT GTGGAGTAAG TGCACATAGC ATGCTGACTT GCTCATCAGT TTGCTGACTG TGCATATTG GGACTGATT AGAACAGAAA CCTCCATTGGA
 CAATAGACCA CACCTCATTG ACGTAAATCG TACGACTGAA CGAGTAGTCA AACGCTGTCA CCTGACIRAA TCTTGTCTT GAGGTAACT
 163 V I W C G V S A J S M L T C S S V L H S G N F G T D E Q K L H N N
 tseI
 fnu4HI/bsoFI
 sau3AI sfcI
 mboI/ndeII [dam-]
 dpnII [dar-]
 dpnI [dam+]
 bbvI
 bclI [dam-]
 hpyCH4V
 avai
 nlaIII pstI [M.H1-]
 hpy188I
 901 ACCCCGAGGA CAAAGGTAT GTGCCTCACA TGATCACTAC TGCAGCAGAA TGGTCTATGT CATTTCCTT CTTGGTCTT TTCCCTGACTT ACATTCGGAA
 TGGGGCCTT GTTCCATA CACGAAGTGT ACTAGTGT ACCAGATACA GTAAANGGA GAAACCAAAGA AAGGACTGAA TGTAGGCACT
 197 P E D K G Y V I H M I T T A A E N S N S F S F F G F F L T Y I R D

thaI
 fnuDI
 acII
 fnu4HI/
 haellI/p
 mcrI pl
 eagI/xmaI
 eaeI ml
 cfrI hi
 osiEI dr
 notI bstUI
 fnu4HI/bso
 acII bshI2

 rboII
 bpuAI tru9I
 bsoI mseI
 bspCNI

 120: AAGGAGACTA TTAACAC CTAAGCCTAT ACTTTTAT CTCAGAAAT AAGCTGAAAG GACTATGAAAG AAAAAGAAAG GGGGGCCGGC
 TTCTCTGAT ATTTCGGTAC TATACGGATA TGAATAATA GAGCTTTA TTTCAGTTT CTTGACTTT TTTCATTTT TTTCATTTT CCGCCGGGGC

 sau96I [M. haellI-]
 haellI/palI
 rwoI styI
 fnu4HI/bsoFI
 bglI [M. haellI-]
 hinfI [M. taqI-]
 sfI ncoI [M. haellI-]
 rmaI taqI sfcI eaeI dsal
 mael salI psI c_rI btgl/bstEII
 bfal hincII/hindII [M. taqI-]
 bsalI accI [M. taqI-] aluI haellI/palI
 hpy188II bspMI hndII acII rlaII

 1301 ACTCTGAGT CGACCTGCAG AAGCTGGCC GCCATGGCC CACTTGTATA TTATGGTAC AAT
 TGAGAATCA GCTGGACGTC TTGAAACCGG CGGTACCGG TTGAACTAAT AACGTCGAAT ATTACCAAT TTAA

hpy188I
 ddeI
 hpy188I

hpyCH4V
 maellI

bbVI psII
 hpyCH4V
 maellI

GSeqEdit, DNA26843 [full], page 7

> Length: 1374

accII (CTMKAC) :	1309
aciII (CCGC) :	110 401 1292 1296 1329
aciII (AACGTT) :	470
acyII (ERCGYC) :	103
afelI (AGGGCT) :	116
afIIII (ACRYGT) :	86 574
aharII (GRCGYC) :	103
aiuII (AGCT) :	361 586 1322 1355
aiw44I (GTGCAC) :	720
aiw5 (GGATC _n NNN) :	55 56 67 68 ^a
apalII (G _n GCAC) :	720
apoI (RAATTY) :	36 909 1097
apyI (CCGGG) :	3
aspHII (GNECWC) :	720
aval (CYCGERG) :	903
avaII (GGGCC) :	6
avalII (ATGCAT) :	412
bamHII (GGATCC) :	55
banI (GGY ₂ CC) :	103
banII (GRGCYC) :	607
bbstII (GAAGACNNNNNN) :	169 677 1203
bbviI (GCAGG) :	275 569 552 842 1353
bcceAI (ACGGCN _n NNNNNN) :	99 ^c
bcI: (TGATCA) :	83 ^c
bfaI: (CTAG) :	62 120 1304
bfrBI (ATGCAT) :	412

bsgII (GCCWNNNGGC) :	43 1328
bnyII (GCCCHC) :	607 720
bpnI (CTGGAG) :	363
bpwII (GAAGACNNNN) :	169 677 1203
bsaII (GRCGYC) :	103
bsaJI (CCNNGG) :	2 18 92 664 804 1332
bsaXI (NNNNNNNNNNACNNNNNNCTCCNNNNNN) :	356 784
bsgI (GTGCAG) :	13 149 576 960
bsh1236I (CCCG) :	87 1297
bsiCI (TTCGAA) :	33
bsiEI (CCRYCG) :	1293
bsiHKA1 (GGGCC) :	726
bsmE (GGGACNNNNNNNN) :	770
bsmI (GAATGCN) :	1138
bsOFI (GCNGC) :	110 275 401 569 652 842 1292 1295 1328 1353
bsp106 (ATCGAT) :	20
bsp1286 (GGGCEC) :	607 720
bspCNI (CTCAGNNNNNN) :	236 455 515 1051 1241
bspDI (ATCGAT) :	28
bspMI (ACCTCC) :	151 161 1313
bsrDI (GCAGNN) :	386 415
bsrI (ACTGGN) :	48 350
bssKI (CCNGG) :	3 92 325
bst4CI (ACNGT) :	356 691 756 1124
bstB1 (TTCGAA) :	33
bstDSI (CCRYGG) :	664 1332
bstE5I (GGATG) :	634
bstN2 (CCNGG) :	3
bstUI (GGCG) :	87 1297
bstY1 (RGATCY) :	55 66

b ⁻ g ⁻ (CCRYGG) :	664 1332
b ⁻ s ⁻ (GCAGTGN) :	958
cacBI (GCNGC) :	106 113 494 729
cfoI (GGCC) :	104 117
cfrI (YGGCCR) :	41 50 111 1293 1326
clai (ATCGAT) :	28
csgf ⁻ (GTAC) :	354 503
ddel (CTNAG) :	236 455 515 725 1051 1241
dgnI (GATC) :	56 67 685 932
gnII (GATC) :	56 67 685 832
drAI (GACNNNNNGTC) :	81 1300
dsAI (CCRYGG) :	664 1332
dsAV (CCNGG) :	3 92 325
eaeI (YGGCCR) :	41 50 111 1293 1326
eagI (CGGCCG) :	1293
earI (CTCTTCNNNN) :	464
ecIXI (CGGCCG) :	1293
eco47III (AGGCT) :	116
eco57 (CTGAAG) :	167 249 452
ecori (GAATT) :	36
ecoriI (CCWGG) :	3
ecory (GATATC) :	1192
ehel (GGGCC) :	103
fnu4HI (GCNGC) :	110 275 401 569 652 842 1292 1295 1328 1353
fruII (CGGG) :	87 1297
fok ⁻ (GGATG) :	634
gsu ⁻ (CTGGAG) :	363
haeII (RGCCY) :	103 116
haeIII (GGCC) :	42 51 112 233 497 1294 1327 1336
hgaI (GACGGC) :	68

kgiAI (GAGCAGC) :	720
khAI (GGCC) :	104 117
hnpI (GGCC) :	104 117
hrcII (GTYRAC) :	1309
hrdII (GTYRAC) :	1309
hndIII (ANGCTT) :	1321
hinfI (GANTC) :	31 457 1047 1300 1307
hiniI (GRCGTC) :	103
hpaII (CCGG) :	53 92 326
hpy188II (TCNNGA) :	166 454 628 687 904 1093 1242
hpyCH4IV (ACNGT) :	61 364 460 549 681 892 895 1001 1150 1193 1303
hpyCH4IV (ACNGT) :	356 691 756 1124
hpyCH4IV (ACGT) :	471
hpyCH4IV (TCGA) :	14 150 301 413 571 651 721 753 841 961 1316 1352
kasI (GCGGCC) :	103
ksq632I (CTCTTCNNNN) :	464
maelI (CTAG) :	62 120 1304
maelI (ACGT) :	471
maeII (GTNAC) :	305 345 1127 1366
mbI (GATC) :	56 67 695 832
mbII (GARGA) :	169 464 678 1098 1203
mcrlI (CGRYCG) :	1293
mluI (ACGGT) :	86
mlvI (GAGTCNNNN) :	457 1300 1307
mnI (CCTC) :	18 59 71 77 235 806 949
mseI (TTAA) :	485 944 975 1111 1211
mallI (CAYNNNNRTG) :	575
mspAII (CYGCKG) :	108
mspI (CCGG) :	53 92 326
myAI (CCGG) :	3

avuI (CCCG) :	87 1297
thcI (GCNNNNNGC) :	43 104 110 1328
nari (GEGGCC) :	103
ncII (CCSGG) :	92 325
ncoI (CCATGG) :	664 1332
ndeII (GATC) :	56 67 685 832
nlaIII (CATG) :	449 575 665 730 829 938 1158 1333
nlaIV (GGNNCC) :	55 103 798
notII (GGGCCGC) :	1292
nsII (ATGCAT) :	412
nspBII (CMGCKG) :	108
nspHII (RCATGY) :	574 729
nspI (RCATGY) :	574 729
paiI (GGCC) :	42 51 112 233 497 1294 1327 1336
pcII (ACATGT) :	574
plet (GAETCNNN) :	457 1300 1307
ppuI (ATGCAT) :	412
psII (TTATAA) :	436 1358
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rmaI (CTAG) :	62 120 1304
rsarI (GTAC) :	354 503
salI (GTCGAC) :	1309
sau3A- (GATC) :	56 67 685 832
sau96- (GGNCC) :	6 1336
scrFI (CCNGG) :	3 92 325
sfcI (CTRYAG) :	300 840 1315
sfiI (GGCCNNNNGGCC) :	42 1327
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smo ⁻ (GTGCCAC) :	720
sph ⁻ (GCATGCC) :	729
ssp ⁻ (AATATT) :	395 1027
stu ⁻ (ASGCCCT) :	232
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not found:

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MOLECULAR BIOLOGY OF
THE CELL

fourth edition

Bruce Alberts

Alexander Johnson

Julian Lewis

Martin Raff

Keith Roberts

Peter Walter

 **Garland Science**
Taylor & Francis Group

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Bruce Alberts received his Ph.D. from Harvard University and is President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California, San Francisco. **Alexander Johnson** received his Ph.D. from Harvard University and is a Professor of Microbiology and Immunology at the University of California, San Francisco. **Julian Lewis** received his D.Phil. from the University of Oxford and is a Principal Scientist at the Imperial Cancer Research Fund, London.

Martin Raff received his M.D. from McGill University and is at the Medical Research Council Laboratory for Molecular Cell Biology and Cell Biology Unit and in the Biology Department at University College London. **Keith Roberts** received his Ph.D. from the University of Cambridge and is Associate Research Director at the John Innes Centre, Norwich. **Peter Walter** received his Ph.D. from The Rockefeller University in New York and is Professor and Chairman of the Department of Biochemistry and Biophysics at the University of California, San Francisco, and an Investigator of the Howard Hughes Medical Institute.

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Back cover In 1967, the British artist Peter Blake created a design classic. Nearly 35 years later Nigel Orme (illustrator), Richard Denyer (photographer), and the authors have together produced an affectionate tribute to Mr Blake's image. With its gallery of icons and influences, its assembly created almost as much complexity, intrigue and mystery as the original. *Drosophila*, *Arabidopsis*, Dolly and the assembled company tempt you to dip inside where, as in the original, "a splendid time is guaranteed for all." (Gunter Blobel, courtesy of The Rockefeller University; Marie Curie, Keystone Press Agency Inc; Darwin bust, by permission of the President and Council of the Royal Society; Rosalind Franklin, courtesy of Cold Spring Harbor Laboratory Archives; Dorothy Hodgkin, © The Nobel Foundation, 1964; James Joyce, etching by Peter Blake; Robert Johnson, photo booth self-portrait early 1930s, © 1986 Delta Haze Corporation all rights reserved, used by permission; Albert L. Lehninger, (unidentified photographer) courtesy of The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions; Linus Pauling, from Ava Helen and Linus Pauling Papers, Special Collections, Oregon State University; Nicholas Poussin, courtesy of ArtToday.com; Barbara McClintock, © David Micklos, 1983; Andrei Sakharov, courtesy of Elena Bonner; Frederick Sanger, © The Nobel Foundation, 1958.)

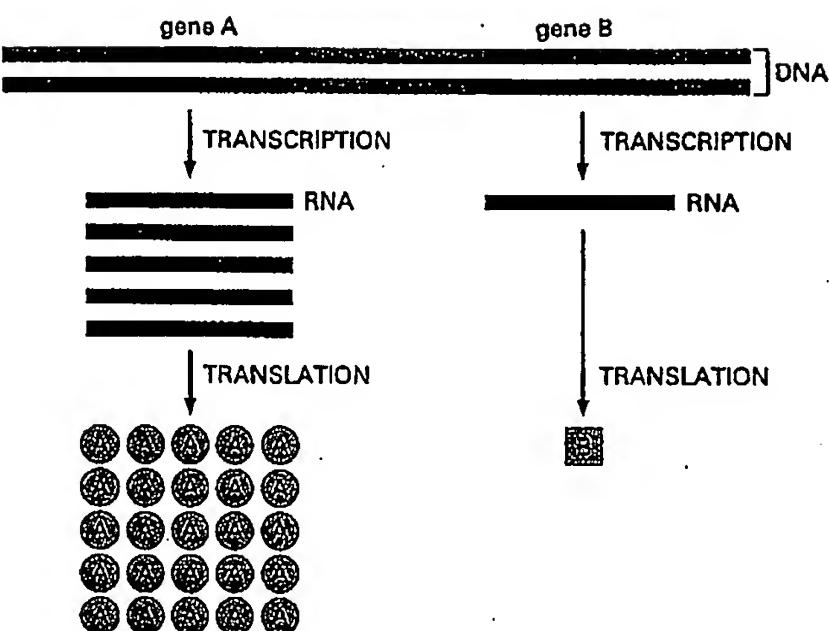


Figure 6-3 Genes can be expressed with different efficiencies. Gene A is transcribed and translated much more efficiently than gene B. This allows the amount of protein A in the cell to be much greater than that of protein B.

FROM DNA TO RNA

Transcription and translation are the means by which cells read out, or express, the genetic instructions in their genes. Because many identical RNA copies can be made from the same gene, and each RNA molecule can direct the synthesis of many identical protein molecules, cells can synthesize a large amount of protein rapidly when necessary. But each gene can also be transcribed and translated with a different efficiency, allowing the cell to make vast quantities of some proteins and tiny quantities of others (Figure 6-3). Moreover, as we see in the next chapter, a cell can change (or regulate) the expression of each of its genes according to the needs of the moment—most obviously by controlling the production of its RNA.

Portions of DNA Sequence Are Transcribed into RNA

The first step a cell takes in reading out a needed part of its genetic instructions is to copy a particular portion of its DNA nucleotide sequence—a gene—into an RNA nucleotide sequence. The information in RNA, although copied into another chemical form, is still written in essentially the same language as it is in DNA—the language of a nucleotide sequence. Hence the name **transcription**.

Like DNA, RNA is a linear polymer made of four different types of nucleotide subunits linked together by phosphodiester bonds (Figure 6-4). It differs from DNA chemically in two respects: (1) the nucleotides in RNA are *ribonucleotides*—that is, they contain the sugar ribose (hence the name *ribonucleic acid*) rather than deoxyribose; (2) although, like DNA, RNA contains the bases adenine (A), guanine (G), and cytosine (C), it contains the base uracil (U) instead of the thymine (T) in DNA. Since U, like T, can base-pair by hydrogen-bonding with A (Figure 6-5), the complementary base-pairing properties described for DNA in Chapters 4 and 5 apply also to RNA (in RNA, G pairs with C, and A pairs with U). It is not uncommon, however, to find other types of base pairs in RNA: for example, G pairing with U occasionally.

Despite these small chemical differences, DNA and RNA differ quite dramatically in overall structure. Whereas DNA always occurs in cells as a double-stranded helix, RNA is single-stranded. RNA chains therefore fold up into a variety of shapes, just as a polypeptide chain folds up to form the final shape of a protein (Figure 6-6). As we see later in this chapter, the ability to fold into complex three-dimensional shapes allows some RNA molecules to have structural and catalytic functions.

Transcription Produces RNA Complementary to One Strand of DNA

All of the RNA in a cell is made by DNA transcription, a process that has certain similarities to the process of DNA replication discussed in Chapter 5.

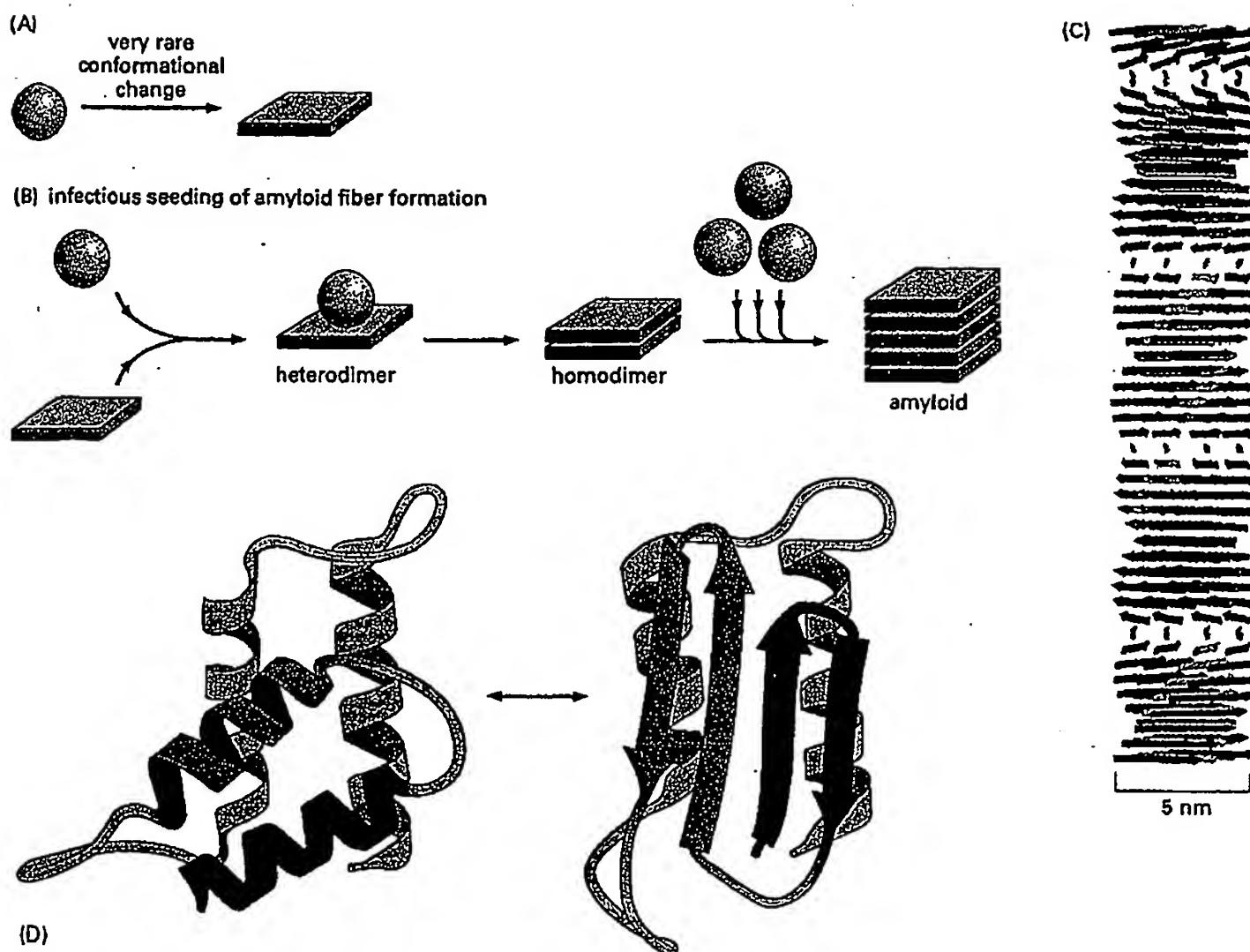


Figure 6-89 Protein aggregates that cause human disease. (A) Schematic illustration of the type of conformational change in a protein that produces material for a cross-beta filament. (B) Diagram illustrating the self-infectious nature of the protein aggregation that is central to prion diseases. PrP is highly unusual because the misfolded version of the protein, called PrP^* , induces the normal PrP protein it contacts to change its conformation, as shown. Most of the human diseases caused by protein aggregation are caused by the overproduction of a variant protein that is especially prone to aggregation, but because this structure is not infectious in this way, it cannot spread from one animal to another. (C) Drawing of a cross-beta filament, a common type of protease-resistant protein aggregate found in a variety of human neurological diseases. Because the hydrogen-bond interactions in a β sheet form between polypeptide backbone atoms (see Figure 3-9), a number of different abnormally folded proteins can produce this structure. (D) One of several possible models for the conversion of PrP to PrP^* , showing the likely change of two α -helices into four β -strands. Although the structure of the normal protein has been determined accurately, the structure of the infectious form is not yet known with certainty because the aggregation has prevented the use of standard structural techniques. (C, courtesy of Louise Serpell, adapted from M. Sunde et al., *J. Mol. Biol.* 273:729–739, 1997; D, adapted from S.B. Prusiner, *Trends Biochem. Sci.* 21:482–487, 1996.)

animals and humans. It can be dangerous to eat the tissues of animals that contain PrP^* , as witnessed most recently by the spread of BSE (commonly referred to as the “mad cow disease”) from cattle to humans in Great Britain.

Fortunately, in the absence of PrP^* , PrP is extraordinarily difficult to convert to its abnormal form. Although very few proteins have the potential to misfold into an infectious conformation, a similar transformation has been discovered to be the cause of an otherwise mysterious “protein-only inheritance” observed in yeast cells.

There Are Many Steps From DNA to Protein

We have seen so far in this chapter that many different types of chemical reactions are required to produce a properly folded protein from the information contained in a gene (Figure 6-90). The final level of a properly folded protein in a cell therefore depends upon the efficiency with which each of the many steps is performed.

We discuss in Chapter 7 that cells have the ability to change the levels of their proteins according to their needs. In principle, any or all of the steps in Fig-

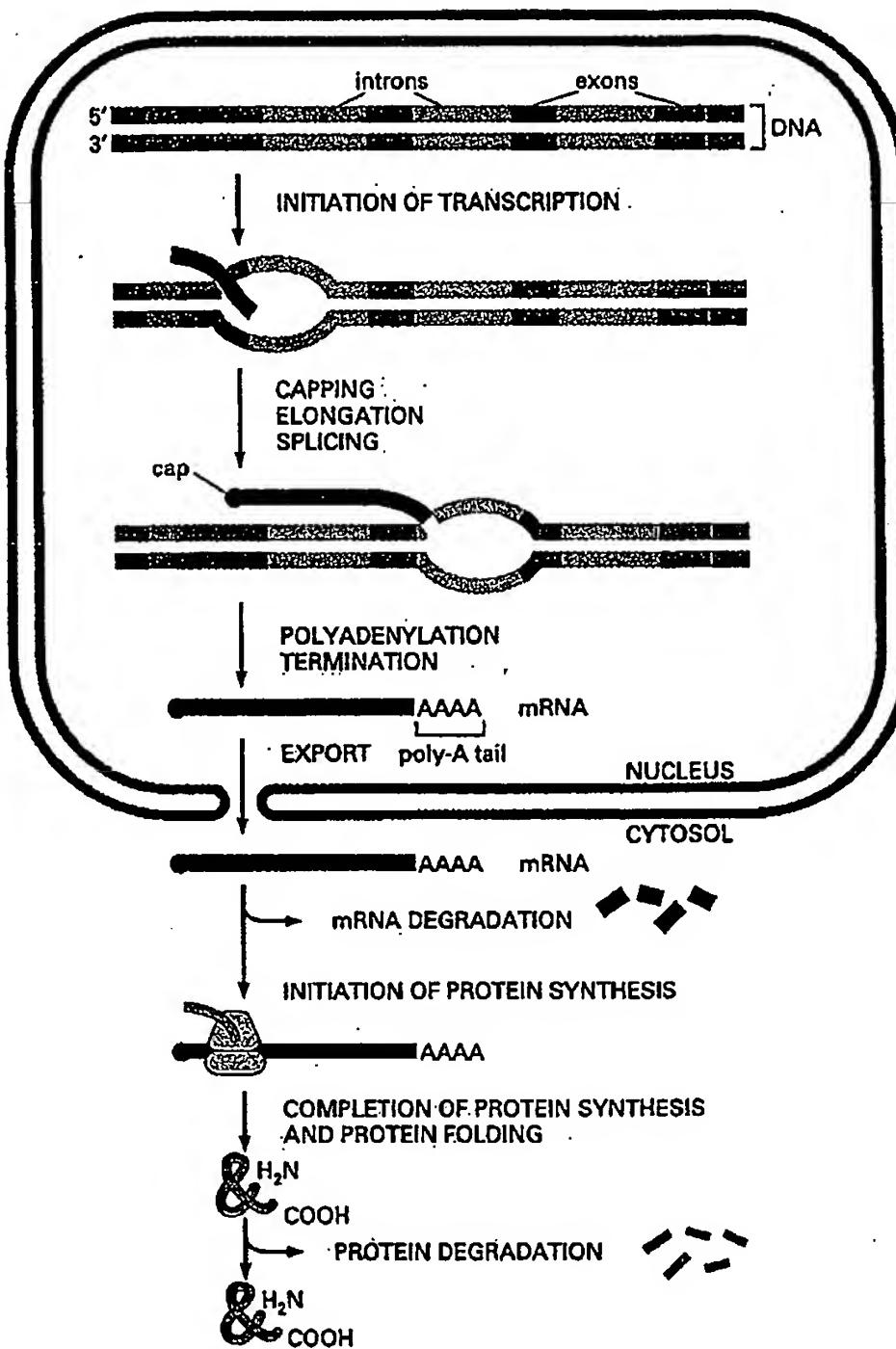


Figure 6-90 The production of a protein by a eucaryotic cell. The final level of each protein in a eucaryotic cell depends upon the efficiency of each step depicted.

ure 6-90) could be regulated by the cell for each individual protein. However, as we shall see in Chapter 7, the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes. This makes sense, inasmuch as the most efficient way to keep a gene from being expressed is to block the very first step—the transcription of its DNA sequence into an RNA molecule.

Summary

The translation of the nucleotide sequence of an mRNA molecule into protein takes place in the cytoplasm on a large ribonucleoprotein assembly called a ribosome. The amino acids used for protein synthesis are first attached to a family of tRNA molecules, each of which recognizes, by complementary base-pair interactions, particular sets of three nucleotides in the mRNA (codons). The sequence of nucleotides in the mRNA is then read from one end to the other in sets of three according to the genetic code.

To initiate translation, a small ribosomal subunit binds to the mRNA molecule at a start codon (AUG) that is recognized by a unique initiator tRNA molecule. A large ribosomal subunit binds to complete the ribosome and begin the elongation phase of protein synthesis. During this phase, aminoacyl tRNAs—each bearing a specific amino acid bind sequentially to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. Each amino acid is added to the C-terminal end of the growing polypeptide by means of a cycle of three sequential

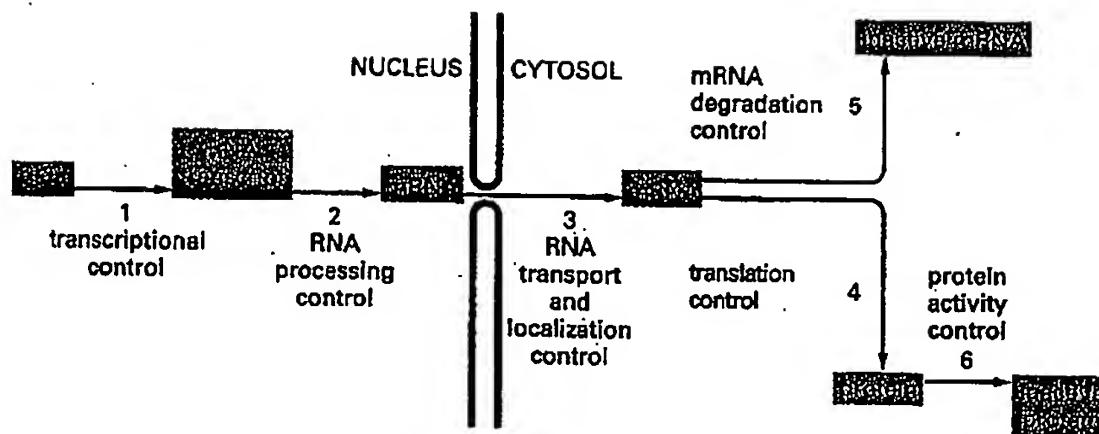


Figure 7-5 Six steps at which eukaryotic gene expression can be controlled. Controls that operate at steps 1 through 5 are discussed in this chapter. Step 6, the regulation of protein activity, includes reversible activation or inactivation by protein phosphorylation (discussed in Chapter 3) as well as irreversible inactivation by proteolytic degradation (discussed in Chapter 6).

Gene Expression Can Be Regulated at Many of the Steps in the Pathway from DNA to RNA to Protein

If differences among the various cell types of an organism depend on the particular genes that the cells express, at what level is the control of gene expression exercised? As we saw in the last chapter, there are many steps in the pathway leading from DNA to protein, and all of them can in principle be regulated. Thus a cell can control the proteins it makes by (1) controlling when and how often a given gene is transcribed (transcriptional control), (2) controlling how the RNA transcript is spliced or otherwise processed (RNA processing control), (3) selecting which completed mRNAs in the cell nucleus are exported to the cytosol and determining where in the cytosol they are localized (RNA transport and localization control), (4) selecting which mRNAs in the cytoplasm are translated by ribosomes (translational control), (5) selectively destabilizing certain mRNA molecules in the cytoplasm (mRNA degradation control), or (6) selectively activating, inactivating, degrading, or compartmentalizing specific protein molecules after they have been made (protein activity control) (Figure 7-5).

For most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 7-5, only transcriptional control ensures that the cell will not synthesize superfluous intermediates. In the following sections we discuss the DNA and protein components that perform this function by regulating the initiation of gene transcription. We shall return at the end of the chapter to the additional ways of regulating gene expression.

Summary

The genome of a cell contains in its DNA sequence the information to make many thousands of different protein and RNA molecules. A cell typically expresses only a fraction of its genes, and the different types of cells in multicellular organisms arise because different sets of genes are expressed. Moreover, cells can change the pattern of genes they express in response to changes in their environment, such as signals from other cells. Although all of the steps involved in expressing a gene can in principle be regulated, for most genes the initiation of RNA transcription is the most important point of control.

DNA-BINDING MOTIFS IN GENE REGULATORY PROTEINS

How does a cell determine which of its thousands of genes to transcribe? As mentioned briefly in Chapters 4 and 6, the transcription of each gene is controlled by a regulatory region of DNA relatively near the site where transcription begins. Some regulatory regions are simple and act as switches that are thrown by a single signal. Many others are complex and act as tiny microprocessors, responding to a variety of signals that they interpret and integrate to switch the neighboring gene on or off. Whether complex or simple, these switching devices

occur in the germ line, the cell lineage that gives rise to sperm or eggs. Most of the DNA in vertebrate germ cells is inactive and highly methylated. Over long periods of evolutionary time, the methylated CG sequences in these inactive regions have presumably been lost through spontaneous deamination events that were not properly repaired. However promoters of genes that remain active in the germ cell lineages (including most housekeeping genes) are kept unmethylated, and therefore spontaneous deaminations of Cs that occur within them can be accurately repaired. Such regions are preserved in modern day vertebrate cells as CG islands. In addition, any mutation of a CG sequence in the genome that destroyed the function or regulation of a gene in the adult would be selected against, and some CG islands are simply the result of a higher than normal density of critical CG sequences.

The mammalian genome contains an estimated 20,000 CG islands. Most of the islands mark the 5' ends of transcription units and thus, presumably, of genes. The presence of CG islands often provides a convenient way of identifying genes in the DNA sequences of vertebrate genomes.

Summary

The many types of cells in animals and plants are created largely through mechanisms that cause different genes to be transcribed in different cells. Since many specialized animal cells can maintain their unique character through many cell division cycles and even when grown in culture, the gene regulatory mechanisms involved in creating them must be stable once established and heritable when the cell divides. These features endow the cell with a memory of its developmental history. Bacteria and yeasts provide unusually accessible model systems in which to study gene regulatory mechanisms. One such mechanism involves a competitive interaction between two gene regulatory proteins, each of which inhibits the synthesis of the other; this can create a flip-flop switch that switches a cell between two alternative patterns of gene expression. Direct or indirect positive feedback loops, which enable gene regulatory proteins to perpetuate their own synthesis, provide a general mechanism for cell memory. Negative feedback loops with programmed delays form the basis for cellular clocks.

In eucaryotes the transcription of a gene is generally controlled by combinations of gene regulatory proteins. It is thought that each type of cell in a higher eucaryotic organism contains a specific combination of gene regulatory proteins that ensures the expression of only those genes appropriate to that type of cell. A given gene regulatory protein may be active in a variety of circumstances and typically is involved in the regulation of many genes.

In addition to diffusible gene regulatory proteins, inherited states of chromatin condensation are also used by eucaryotic cells to regulate gene expression. An especially dramatic case is the inactivation of an entire X chromosome in female mammals. In vertebrates DNA methylation also functions in gene regulation, being used mainly as a device to reinforce decisions about gene expression that are made initially by other mechanisms. DNA methylation also underlies the phenomenon of genomic imprinting in mammals, in which the expression of a gene depends on whether it was inherited from the mother or the father.

POSTTRANSCRIPTIONAL CONTROLS

In principle, every step required for the process of gene expression could be controlled. Indeed, one can find examples of each type of regulation, although any one gene is likely to use only a few of them. Controls on the initiation of gene transcription are the predominant form of regulation for most genes. But other controls can act later in the pathway from DNA to protein to modulate the amount of gene product that is made. Although these posttranscriptional controls, which operate after RNA polymerase has bound to the gene's promoter and begun RNA synthesis, are less common than transcriptional control, for many genes they are crucial.

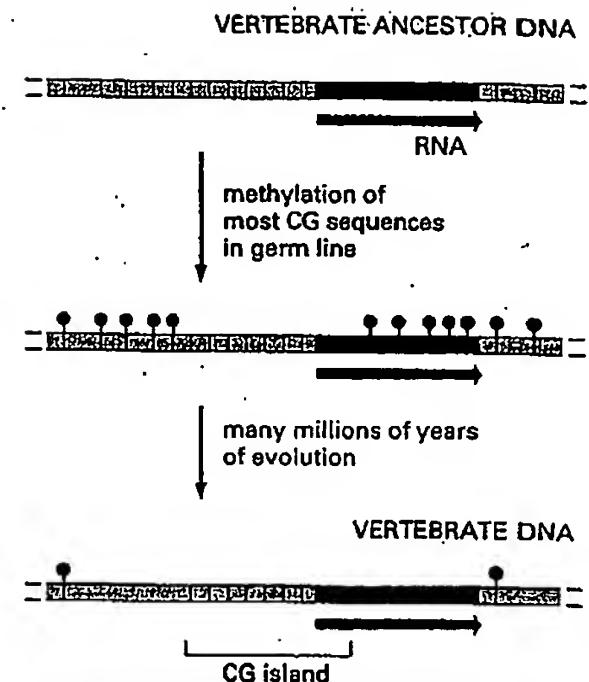
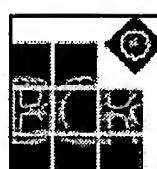


Figure 7-86 A mechanism to explain both the marked overall deficiency of CG sequences and their clustering into CG islands in vertebrate genomes. A black line marks the location of a CG dinucleotide in the DNA sequence, while a red "lollipop" indicates the presence of a methyl group on the CG dinucleotide. CG sequences that lie in regulatory sequences of genes that are transcribed in germ cells are unmethylated and therefore tend to be retained in evolution. Methylated CG sequences, on the other hand, tend to be lost through deamination of 5-methyl C to T, unless the CG sequence is critical for survival.

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Open Access**Prostate stem cell antigen (PSCA) expression in
human prostate cancer tissues and its potential role
in prostate carcinogenesis and progression of prostate
cancer****Zhao Zhigang**¹ and **Shen Wenlv**² ¹Department of Urology, Shantou University Medical College, Shantou, Guangdong, China²Department of Urology, No 2. Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China*World Journal of Surgical Oncology* 2004, **2**:13 doi:10.1186/1477-7819-2-13The electronic version of this article is the complete one and can be found online at:
<http://www.wjso.com/content/2/1/13>**Received** 30 March 2004**Accepted** 10 May 2004**Published** 10 May 2004

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Keywords: Prostate, Neoplasm, Prostate stem cell antigen (PSCA)**Outline****Abstract****Abstract****Background**

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Prostate stem cell antigen (PSCA) is a recently defined homologue of the Thy-1/Ly-6 family of glycosylphosphatidylinositol (GPI)-anchored cell surface antigens. The purpose of the present study was to examine the expression status of PSCA protein and mRNA in clinical specimens of human prostate cancer (Pca) and to validate it as a potential molecular target for diagnosis and treatment of Pca.

Materials and Methods

Immunohistochemical (IHC) and *in situ* hybridization (ISH) analyses of PSCA expression were simultaneously performed on paraffin-embedded sections from 20 benign prostatic hyperplasia (BPH), 20 prostatic Intraepithelial neoplasm (PIN) and 48 prostate cancer (Pca) tissues, including 9 androgen-independent prostate cancers. The level of PSCA expression was semiquantitatively scored by assessing both the percentage and intensity of PSCA-positive staining cells in the specimens. Then compared PSCA expression between BPH, PIN and Pca tissues and analysed the correlations of PSCA expression level with pathological grade, clinical stage and

progression to androgen-independence in Pca.

Results

In BPH and low grade PIN, PSCA protein and mRNA staining were weak or negative and less intense and uniform than that seen in HGPIN and Pca. There were moderate to strong PSCA protein and mRNA expression in 8 of 11 (72.7%) HGPIN and in 40 of 48 (83.4%) Pca specimens examined by IHC and ISH analyses, with statistical significance compared with BPH (20%) and low grade PIN (22.2%) samples ($p < 0.05$, respectively). The expression level of PSCA increased with high Gleason grade, advanced stage and progression to androgen-independence ($p < 0.05$, respectively). In addition, IHC and ISH staining showed a high degree of correlation between PSCA protein and mRNA overexpression.

Conclusions

Our data demonstrate that PSCA as a new cell surface marker is overexpressed by a majority of human Pca. PSCA expression correlates positively with adverse tumor characteristics, such as increasing pathological grade (poor cell differentiation), worsening clinical stage and androgen-independence, and speculatively with prostate carcinogenesis. PSCA protein overexpression results from upregulated transcription of PSCA mRNA. PSCA may have prognostic utility and may be a promising molecular target for diagnosis and treatment of Pca.

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Prostate cancer (Pca) is the second leading cause of cancer-related death in American men and is becoming a common cancer increasing in China. Despite recently great progress in the diagnosis and management of localized disease, there continues to be a need for new diagnostic markers that can accurately discriminate between indolent and aggressive variants of Pca. There also continues to be a need for the identification and characterization of potential new therapeutic targets on Pca cells. Current diagnostic and therapeutic modalities for recurrent and metastatic Pca have been limited by a lack of specific target antigens of Pca.

Although a number of prostate-specific genes have been identified (i.e. prostate specific antigen, prostatic acid phosphatase, glandular kallikrein 2), the majority of these are secreted proteins not ideally suited for many immunological strategies. So, the identification of new cell surface antigens is critical to the development of new diagnostic and therapeutic approaches to the management of Pca.

Reiter RE et al [1] reported the identification of prostate stem cell antigen (PSCA), a cell surface antigen that is predominantly prostate specific. The PSCA gene encodes a 123 amino acid glycoprotein, with 30% homology to stem cell antigen 2 (Sca 2). Like Sca-2, PSCA also belongs to a member of the Thy-1/Ly-6 family and is anchored by a glycosylphosphatidylinositol (GPI) linkage. mRNA *in situ* hybridization (ISH) localized PSCA expression in normal prostate to the basal cell epithelium, the putative stem cell compartment of prostatic epithelium, suggesting that PSCA may be a marker of prostate stem/progenitor cells.

In order to examine the status of PSCA protein and mRNA expression in human Pca

and validate it as a potential diagnostic and therapeutic target for Pca, we used immunohistochemistry (IHC) and *in situ* hybridization (ISH) simultaneously, and conducted PSCA protein and mRNA expression analyses in paraffin-embedded tissue specimens of benign prostatic hyperplasia (BPH, n = 20), prostate intraepithelial neoplasm (PIN, n = 20) and prostate cancer (Pca, n = 48). Furthermore, we evaluated the possible correlation of PSCA expression level with Pca tumorigenesis, grade, stage and progression to androgen-independence.

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Materials and methods

Tissue samples

All of the clinical tissue specimens studied herein were obtained from 80 patients of 57–84 years old by prostatectomy, transurethral resection of prostate (TURP) or biopsies. The patients were classified as 20 cases of BPH, 20 cases of PIN, 40 cases of primary Pca, including 9 patients with recurrent Pca and a history of androgen ablation therapy (orchietomy and/or hormonal therapy), who were referred to as androgen-independent prostate cancers. Eight specimens were harvested from these androgen-independent Pca patients prior to androgen ablation treatment. Each tissue sample was cut into two parts, one was fixed in 10% formalin for IHC and the other treated with 4% paraformaldehyde/0.1 M PBS PH 7.4 in 0.1% DEPC for 1 h for ISH analysis, and then embedded in paraffin. All paraffin blocks examined were then cut into 5 μ m sections and mounted on the glass slides specific for IHC and ISH respectively in the usual fashion. H&E-stained section of each Pca was evaluated and assigned a Gleason score by the experienced urological pathologist at our institution based on the criteria of Gleason score [2]. The Gleason sums are summarized in Table 1. Clinical staging was performed according to Jewett-whitmore-prout staging system, as shown in Table 2. In the category of PIN, we graded the specimens into two groups, i.e. low grade PIN (grade I – II) and high grade PIN (HGPIN, grade III) on the basis of literatures [3,4].

Immunohistochemical (IHC) analysis

Briefly, tissue sections were deparaffinized, dehydrated, and subjected to microwaving in 10 mmol/L citrate buffer, PH 6.0 (Boshide, Wuhan, China) in a 900 W oven for 5 min to induce epitope retrieval. Slides were allowed to cool at room temperature for 30 min. A primary mouse antibody specific to human PSCA (Boshide, Wuhan, China) with a 1:100 dilution was applied to incubate with the slides at room temperature for 2 h. Labeling was detected by sequentially adding biotinylated secondary antibodies and strepavidin-peroxidase, and localized using 3,3'-diaminobenzidine reaction. Sections were then counterstained with hematoxylin. Substitution of the primary antibody with phosphate-buffered-saline (PBS) served as a negative-staining control.

mRNA *in situ* hybridization (ISH)

Five- μ m-thick tissue sections were deparaffinized and dehydrated, then digested in pepsin solution (4 mg/ml in 3% citric acid) for 20 min at 37.5°C, and further processed for ISH. Digoxigenin-labeled sense and antisense human PSCA RNA probes (obtained from Boshide, Wuhan, China) were hybridized to the sections at

48°C overnight. The posthybridization wash with a high stringency was performed sequentially at 37°C in 2 × standard saline citrate (SSC) for 10 min, in 0.5 × SSC for 15 min and in 0.2 × SSC for 30 min. The slides were then incubated to biotinylated mouse anti-digoxigenin antibody at 37.5°C for 1 h followed by washing in 1 × PBS for 20 min at room temperature, and then to strepavidin-peroxidase at 37.5°C for 20 min followed by washing in 1 × PBS for 15 min at room temperature. Subsequently, the slides were developed with diaminobenzidine and then counterstained with hematoxylin to localize the hybridization signals. Sections hybridized with the sense control probes routinely did not show any specific hybridization signal above background. All slides were hybridized with PBS to substitute for the probes as a negative control.

Scoring methods

To determine the correlation between the results of PSCA immunostaining and mRNA *in situ* hybridization, the same scoring manners are taken in the present study for PSCA protein staining by IHC and PSCA mRNA staining by ISH. Each slide was read and scored by two independently experienced urological pathologists using Olympus BX-41 light microscopes. The evaluation was done in a blinded fashion. For each section, five areas of similar grade were analyzed semiquantitatively for the fraction of cells staining. Fifty percent of specimens were randomly chosen and rescored to determine the degree of interobserver and intraobserver concordance. There was greater than 95% intra- and interobserver agreement.

The intensity of PSCA expression evaluated microscopically was graded on a scale of 0 to 3+ with 3 being the highest expression observed (0, no staining; 1+, mildly intense; 2+, moderately intense; 3+, severely intense). The staining density was quantified as the percentage of cells staining positive for PSCA with the primary antibody or hybridization probe, as follows: 0 = no staining; 1 = positive staining in <25% of the sample; 2 = positive staining in 25%–50% of the sample; 3 = positive staining in >50% of the sample. Intensity score (0 to 3+) was multiplied by the density score (0–3) to give an overall score of 0–9 [1,5]. In this way, we were able to differentiate specimens that may have had focal areas of increased staining from those that had diffuse areas of increased staining [6]. The overall score for each specimen was then categorically assigned to one of the following groups: 0 score, negative expression; 1–2 scores, weak expression; 3–6 scores, moderate expression; 9 score, strong expression.

Statistical analysis

Intensity and density of PSCA protein and mRNA expression in BPH, PIN and Pca tissues were compared using the Chi-square and Student's *t*-test. Univariate associations between PSCA expression and Gleason score, clinical stage and progression to androgen-independence were calculated using Fisher's Exact Test. For all analyses, *p* < 0.05 was considered statistically significant.

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Figure 1
Representatives of PSCA IHC and ISH staining in Pca (A. IHC staining, B. ISH staining, $\times 200$ magnification)

In general, PSCA protein and mRNA were expressed weakly in individual samples of BPH. Some areas of prostate expressed weak levels (composite score 1–2), whereas other areas were completely negative (composite score 0). Four cases (20%) of BPH had moderate expression of PSCA protein and mRNA (composite score 4–6) by IHC and ISH. In 2/20 (10%) BPH specimens, PSCA mRNA expression was moderate (composite score 3–6), but PSCA protein expression was weak (composite score 2) in one and negative (composite score 0) in the other. PSCA expression was localized to the basal and secretory epithelial cells, and prostatic stroma was almost negative staining for PSCA protein and mRNA in all cases examined.

PSCA expression in PIN

In this study, we detected weak or negative expression of PSCA protein and mRNA (≤ 2 scores) in 7 of 9 (77.8%) low grade PIN and in 2 of 11 (18.2%) HGPIN, and moderate expression (3–6 scores) in the rest 2 low grade PIN and 5 of 11 (45.5%) HGPIN. One HGPIN with moderate PSCA mRNA expression (6 score) was found weak staining for PSCA protein (2 score) by IHC. Strong PSCA protein and mRNA expression (9 score) were detected in the remaining 3 of 11 (27.3%) HGPIN. There was a statistically significant difference of PSCA protein and mRNA expression levels observed between HGPIN and BPH ($p < 0.05$), but no statistical difference reached between low grade PIN and BPH ($p > 0.05$).

PSCA expression in Pca

In order to determine if PSCA protein and mRNA can be detected in prostate cancers and if PSCA expression levels are increased in malignant compared with benign glands, Forty-eight paraffin-embedded Pca specimens were analysed by IHC and ISH. It was shown that 19 of 48 (39.6%) Pca samples stained very strongly for PSCA protein and mRNA with a score of 9 and another 21 (43.8%) specimens displayed moderate staining with scores of 4–6 (Figure 1). In addition, 4 specimens with moderate to strong PSCA mRNA expression (scores of 4–9) had weak protein staining (a score of 2) by IHC analyses. Overall, Pca expressed a significantly higher level of PSCA protein and mRNA than any other specimen category in this study ($p < 0.05$, compared with BPH and PIN respectively). The result demonstrates that PSCA protein and mRNA are overexpressed by a majority of human Pca.

Correlation of PSCA expression with Gleason score in Pca

Using the semi-quantitative scoring method as described in Materials and Methods, we compared the expression level of PSCA protein and mRNA with Gleason grade of Pca, as shown in Table 1. Prostate adenocarcinomas were graded by Gleason score as 2–4 scores = well-differentiation, 5–7 scores = moderate-differentiation and 8–10 scores = poor-differentiation [7]. Seventy-two percent of Gleason scores 8–10 prostate cancers had very strong staining of PSCA compared to 21% with Gleason scores 5–7 and 17% with 2–4 respectively, demonstrating that poorly differentiated Pca had significantly stronger expression of PSCA protein and mRNA than moderately and well differentiated tumors ($p < 0.05$). As depicted in Figure 1, IHC and ISH analyses showed that PSCA protein and mRNA expression in several cases of poorly differentiated Pca were particularly prominent, with more intense and uniform staining. The results indicate that PSCA expression increases significantly with higher tumor grade in human Pca.

Correlation of PSCA expression with clinical stage in Pca

With regards to PSCA expression in every stage of Pca, we showed the results in Table 2. Seventy-five percent of locally advanced and node positive cancers (i.e. C-D stages) expressed statistically high levels of PSCA versus 32.5% that were organ confined (i.e. A-B stages) ($p < 0.05$). The data demonstrate that PSCA expression increases significantly with advanced tumor stage in human Pca.

Correlation of PSCA expression with androgen-independent progression of Pca

All 9 specimens of androgen-independent prostate cancers stained positive for PSCA protein and mRNA. Eight specimens were obtained from patients managed prior to androgen ablation therapy. Seven of eight (87.5%) of these androgen-independent prostate cancers were in the strongest staining category (score = 9), compared with three out of eight (37.5%) of patients with androgen-dependent cancers ($p < 0.05$). The results demonstrate that PSCA expression increases significantly with progression to androgen-independence of human Pca.

It is evident from the results above that within a majority of human prostate cancers the level of PSCA protein and mRNA expression correlates significantly with increasing grade, worsening stage and progression to androgen-independence.

Correlation of PSCA immunostaining and mRNA *in situ* hybridization

In all 88 specimens surveyed herein, we compared the results of PSCA IHC staining with mRNA ISH analysis. Positive staining areas and its intensity and density scores evaluated by IHC were identical to those seen by ISH in 79 of 88 (89.8%) specimens (18/20 BPH, 19/20 PIN and 42/48 Pca respectively). Importantly, 27/27 samples with PSCA mRNA composite scores of 0-2, 32/36 samples with scores of 3-6 and 22/24 samples with a score of 9 also had PSCA protein expression scores of 0-2, 3-6 and 9 respectively. However, in 5 samples with PSCA mRNA overall scores of 3-6 and in 2 with scores of 9 there were less or negative PSCA protein expression (i.e. scores of 0-4), suggesting that this may reflect posttranscriptional modification of PSCA or that the epitopes recognized by PSCA mAb may be obscured in some cancers. The data demonstrate that the results of PSCA immunostaining were consistent with those of mRNA ISH analysis, showing a high degree of correlation between PSCA protein and mRNA expression.

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Discussion

PSCA is homologous to a group of cell surface proteins that mark the earliest phase of hematopoietic development. PSCA mRNA expression is prostate-specific in normal male tissues and is highly up-regulated in both androgen-dependent and-independent Pca xenografts (LAPC-4 tumors). We hypothesize that PSCA may play a role in Pca tumorigenesis and progression, and may serve as a target for Pca diagnosis and treatment. In this study, IHC and ISH showed that in general there were weak or absent PSCA protein and mRNA expression in BPH and low grade PIN tissues. However, PSCA protein and mRNA are widely expressed in HGPIN, the putative precursor of invasive Pca, suggesting that up-regulation of PSCA is an

early event in prostate carcinogenesis. Recently, Reiter RE et al [1], using ISH analysis, reported that 97 of 118 (82%) HGPIN specimens stained strongly positive for PSCA mRNA. A very similar finding was seen on mouse PSCA (mPSCA) expression in mouse HGPIN tissues by Tran C. P et al [8]. These data suggest that PSCA may be a new marker associated with transformation of prostate cells and tumorigenesis.

We observed that PSCA protein and mRNA are highly expressed in a large percentage of human prostate cancers, including advanced, poorly differentiated, androgen-independent and metastatic cases. Fluorescence-activated cell sorting and confocal/ immunofluorescent studies demonstrated cell surface expression of PSCA protein in Pca cells [9]. Our IHC expression analysis of PSCA shows not only cell surface but also apparent cytoplasmic staining of PSCA protein in Pca specimens (Figure 1). One possible explanation for this is that anti-PSCA antibody can recognize PSCA peptide precursors that reside in the cytoplasm. Also, it is possible that the positive staining that appears in the cytoplasm is actually from the overlying cell membrane [5]. These data seem to indicate that PSCA is a novel cell surface marker for human Pca.

Our results show that elevated level of PSCA expression correlates with high grade (i.e. poor differentiation), increased tumor stage and progression to androgen-independence of Pca. These findings support the original IHC analyses by Gu Z et al [9], who reported that PSCA protein expressed in 94% of primary Pca and the intensity of PSCA protein expression increased with tumor grade, stage and progression to androgen-independence. Our results also collaborate the recent work of Han KR et al [10], in which the significant association between high PSCA expression and adverse prognostic features such as high Gleason score, seminal vesicle invasion and capsular involvement in Pca was found. It is suggested that PSCA overexpression may be an adverse predictor for recurrence, clinical progression or survival of Pca. Hara H et al [11] used RT-PCR detection of PSA, PSMA and PSCA in 1 ml of peripheral blood to evaluate Pca patients with poor prognosis. The results showed that among 58 PCa patients, each PCR indicated the prognostic value in the hierarchy of PSCA>PSA>PSMA RT-PCR, and extraprostatic cases with positive PSCA PCR indicated lower disease-progression-free survival than those with negative PSCA PCR, demonstrating that PSCA can be used as a prognostic factor. Dubey P et al [12] reported that elevated numbers of PSCA + cells correlate positively with the onset and development of prostate carcinoma over a long time span in the prostates of the TRAMP and PTEN +/- models compared with its normal prostates. Taken together with our present findings, in which PSCA is overexpressed from HGPIN to almost frank carcinoma, it is reasonable and possible to use increased PSCA expression level or increased numbers of PSCA-positive cells in the prostate samples as a prognostic marker to predict the potential onset of this cancer. These data raise the possibility that PSCA may have diagnostic utility or clinical prognostic value in human Pca.

The cause of PSCA overexpression in Pca is not known. One possible mechanism is that it may result from PSCA gene amplification. In humans, PSCA is located on chromosome 8q24.2 [1], which is often amplified in metastatic and recurrent Pca and considered to indicate a poor prognosis [13-15]. Interestingly, PSCA is in close proximity to the c-myc oncogene, which is amplified in >20% of recurrent and metastatic prostate cancers [16,17]. Reiter RE et al [18] reported that PSCA and MYC gene copy numbers were co-amplified in 25% of tumors (five out of twenty), demonstrating that PSCA overexpression is associated with PSCA and MYC coamplification in Pca. Gu Z et al [9] recently reported that in 102 specimens

available to compare the results of PSCA immunostaining with their previous mRNA ISH analysis, 92 (90.2%) had identically positive areas of PSCA protein and mRNA expression. Taken together with our findings, in which we detected moderate to strong expression of PSCA protein and mRNA in 34 of 40 (85%) Pca specimens examined simultaneously by IHC and ISH analyses, it is demonstrated that PSCA protein and mRNA overexpressed in human Pca, and that the increased protein level of PSCA was resulted from the upregulated transcription of its mRNA.

At present, the regulation mechanisms of human PSCA expression and its biological function are yet to be elucidated. PSCA expression may be regulated by multiple factors [18]. Watabe T et al [19] reported that transcriptional control is a major component regulating PSCA expression levels. In addition, induction of PSCA expression may be regulated or mediated through cell-cell contact and protein kinase C (PKC) [20]. Homologues of PSCA have diverse activities, and have themselves been involved in carcinogenesis. Signalling through SCA-2 has been demonstrated to prevent apoptosis in immature thymocytes [21]. Thy-1 is involved in T cell activation and transducts signals through src-like tyrosine kinases [22]. Ly-6 genes have been implicated both in tumorigenesis and in cell-cell adhesion [23-25]. Cell-cell or cell-matrix interaction is critical for local tumor growth and spread to distal sites. From its restricted expression in basal cells of normal prostate and its homology to SCA-2, PSCA may play a role in stem/progenitor cell function, such as self-renewal (i.e. anti-apoptosis) and/or proliferation [1]. Taken together with the results in the present study, we speculate that PSCA may play a role in tumorigenesis and clinical progression of Pca through affecting cell transformation and proliferation. From our results, it is also suggested that PSCA as a new cell surface antigen may have a number of potential uses in the diagnosis, therapy and clinical prognosis of human Pca. PSCA overexpression in prostate biopsies could be used to identify patients at high risk to develop recurrent or metastatic disease, and to discriminate cancers from normal glands in prostatectomy samples. Similarly, the detection of PSCA-overexpressing cells in bone marrow or peripheral blood may identify and predict metastatic progression better than current assays, which identify only PSA-positive or PSMA-positive prostate cells.

In summary, we have shown in this study that PSCA protein and mRNA are maintained in expression from HGPIN through all stages of Pca in a majority of cases, which may be associated with prostate carcinogenesis and correlate positively with high tumor grade (poor cell differentiation), advanced stage and androgen-independent progression. PSCA protein overexpression is due to the upregulation of its mRNA transcription. The results suggest that PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor.

Competing interests

None declared.

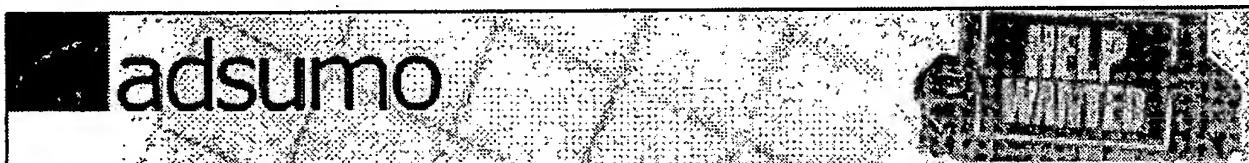
Outline **References**

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1. Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, David E, Wahl M, Nisitani S, Yamashiro J, Le Beau MM, Loda M, Witte ON: **Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer.** *Proc Natl Acad Sci USA* 1998, **95**:1735-1740. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\] \[2\] \[3\] \[4\] \[5\]](#)
2. Gleason DF: **Histologic grading and clinical staging of prostatic carcinoma.** In: *Urologic Pathology: The Prostate* (Edited by: Tannebaum M). Philadelphia, Lea & Febiger 1977, 171-197.
[Return to citation in text: \[1\]](#)
3. Brawer MK: **Prostatic intraepithelial neoplasia: a premalignant lesion.** *Hum Pathol* 1992, **23**:242-248. [PubMed Abstract]
[Return to citation in text: \[1\]](#)
4. Amin MB, Ro JY, Ayala AC: **Prostatic intraepithelial neoplasia: relationship to adenocarcinoma of prostate.** *Pathol Annu* 1994, **29**:1-30. [PubMed Abstract]
[Return to citation in text: \[1\]](#)
5. Amara N, Palapattu GS, Schrage M, Gu Z, Thomas GV, Dorey F, Said J, Reiter RE: **Prostate stem cell antigen is overexpressed in human transitional cell carcinoma.** *Cancer Res* 2001, **61**:4660-4665. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\] \[2\]](#)
6. Hanas JS, Lerner MR, Lightfoot SA, Raczkowski C, Kastens DJ, Brackett DJ, Postier RG: **Expression of the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1) and p53 tumor suppressor in dysplastic progression and adenocarcinoma in Barrett esophagus.** *Cancer (Phila)* 1999, **86**:756-763. [Publisher Full Text]
[Return to citation in text: \[1\]](#)
7. Egevad L, Gramfors T, Karlberg L: **Prognostic value of the Gleason score in prostate cancer.** *BJU Int* 2002, **89**:538-542. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\]](#)
8. Tran CP, Lin C, Yamashiro J, Reiter RE: **Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells.** *Mol Cancer Res* 2002, **1**:113-121. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\]](#)
9. Gu Z, Thomas G, Yamashiro J, Shintaku IP, Dorey F, Raitano A, Witte ON, Said JW, Loda M, Reiter RE: **Prostate stem cell antigen (PSCA) expression increases with high Gleason score, advanced stage and bone metastasis in prostate cancer.** *Oncogene* 2000, **19**:1288-1296. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\] \[2\] \[3\]](#)
10. Han KR, Seligson DB, Liu X, Horvath S, Shintaku PI, Thomas GV, Said JW, Reiter RE: **Prostate stem cell antigen expression is associated with gleason score, seminal vesicle invasion and capsular invasion in prostate cancer.** *J Urol* 2004, **171**:1117-1121. [PubMed Abstract][Publisher Full Text]
[Return to citation in text: \[1\]](#)

11. Hara H, Kasahara T, Kawasaki T, Bilim V, Obara K, Takahashi K, Tomita Y: **Reverse Transcription-Polymerase Chain Reaction Detection of Prostate-specific Antigen, Prostate-specific Membrane Antigen, and Prostate Stem Cell Antigen in One Milliliter of Peripheral Blood.** *Clin Cancer Res* 2002, **8**:1794-1799. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
12. Dubey P, Wu H, Reiter RE, Witte ON: **Alternative pathways to prostate carcinoma activate prostate stem cell antigen expression.** *Cancer Res* 2001, **61**:3256-3261. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
13. Visa korpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP: **Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization.** *Cancer Res* 1995, **55**:342-347. [PubMed Abstract]
Return to citation in text: [1]
14. Sato K, Qian J, Slezak JM, Lieber MM, Bostwick DG, Bergstrahl EJ, Jenkins RB: **Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma.** *J Natl Cancer Inst* 1999, **91**:1574-1580. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
15. Van Den Berg C, Guan XY, Von Hoff D, Jenkins R, Bittner , Griffin C, Kallioniemi O, Visakorpi , McGill , Herath J, Epstein J, Sarosdy M, Meltzer P, Trent J: **DNA sequence amplification in human prostate cancer identified by chromosome microdissection: potential prognostic implications.** *Clin Cancer Res* 1995, **1**:11-18. [PubMed Abstract]
Return to citation in text: [1]
16. Jenkins RB, Qian J, Lieber MM, Bostwick DG: **Detection of c-myc oncogene amplification and chromosomal anomalies in metastatic prostatic carcinoma by fluorescence in situ hybridization.** *Cancer Res* 1997, **57**:524-531. [PubMed Abstract]
Return to citation in text: [1]
17. Nupponen NN, Kakkola L, Koivisto P, Visakorpi T: **Genetic alterations in hormone-refractory recurrent prostate carcinomas.** *Am J Pathol* 1998, **153**:141-148. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
18. Reiter RE, Sato I, Thomas G, Qian J, Gu Z, Watabe T, Loda M, Jenkins RB: **Coamplification of prostate stem cell antigen (PSCA) and MYC in locally advanced prostate cancer.** *Genes Chromosomes Cancer* 2000, **27**:95-103. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1] [2]
19. Watabe T, Lin M, Donjacour AA, Cunha GR, Witte ON, Reiter RE: **Growth, regeneration, and tumorigenesis of the prostate activates the PSCA promoter.** *Proc Natl Acad Sci USA* 2002, **99**:401-406. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]

20. Bahrenberg G, Brauers A, Joost HG, Jakse G: **PSCA expression is regulated by phorbol ester and cell adhesion in the bladder carcinoma cell line RT112.** *Cancer Lett* 2001, **168**:37-43. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
21. Noda S, Kosugi A, Saitoh S, Narumiya S, Hamaoka T: **Protection from anti-TCR/CD3-induced apoptosis in immature thymocytes by a signal through thymic shared antigen-1/stem cell antigen-2.** *J Exp Med* 1996, **183**:2355-2360. [PubMed Abstract]
Return to citation in text: [1]
22. Thomas PM, Samelson LE: **The glycophosphatidylinositol-anchored Thy-1 molecule interacts with the p60^{fyn} protein tyrosine kinase in T cells.** *J Biol Chem* 1992, **267**:12317-12322. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
23. Bamezai A, Rock KL: **Overexpressed Ly-6A.2 mediated cell-cell adhesion by binding a ligand expressed on lymphoid cells.** *Proc Natl Acad Sci USA* 1995, **92**:4294-4298. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]
24. Katz BZ, Eshel R, Sagi-Assif O, Witz IP: **An association between high Ly-6A/E expression on tumor cells and a highly malignant phenotype.** *Int J Cancer* 1994, **59**:684-691. [PubMed Abstract]
Return to citation in text: [1]
25. Brakenhoff RH, Gerretsen M, Knippels EM, van Dijk M, van Essen H, Weghuis DO, Sinke RJ, Snow GB, van Dongen GA: **The human E48 antigen, highly homologous to the murine Ly-6 antigen ThB, is a GPI-anchored molecule apparently involved in keratinocyte cell-cell adhesion.** *J Cell Biol* 1995, **129**:1677-1689. [PubMed Abstract][Publisher Full Text]
Return to citation in text: [1]



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Review

Translation Initiation in Cancer: A Novel Target for Therapy¹

Funda Meric² and Kelly K. Hunt

Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

Translation initiation is regulated in response to nutrient availability and mitogenic stimulation and is coupled with cell cycle progression and cell growth. Several alterations in translational control occur in cancer. Variant mRNA sequences can alter the translational efficiency of individual mRNA molecules, which in turn play a role in cancer biology. Changes in the expression or availability of components of the translational machinery and in the activation of translation through signal transduction pathways can lead to more global changes, such as an increase in the overall rate of protein synthesis and translational activation of the mRNA molecules involved in cell growth and proliferation. We review the basic principles of translational control, the alterations encountered in cancer, and selected therapies targeting translation initiation to help elucidate new therapeutic avenues.

Introduction

The fundamental principle of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells. With the advent of cDNA array technology, most efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable either to DNA amplification or to differences in transcription. Gene expression is quite complicated, however, and is also regulated at the level of mRNA stability, mRNA translation, and protein stability.

The power of translational regulation has been best recognized among developmental biologists, because transcription does not occur in early embryogenesis in eukaryotes. For example, in *Xenopus*, the period of transcriptional quiescence continues until the embryo reaches midblastula transition, the 4000-cell stage. Therefore, all necessary mRNA molecules are transcribed during oogenesis and stockpiled in a translationally inactive, masked form. The mRNA are translationally activated at appropriate times during oocyte maturation, fertilization, and

early embryogenesis and thus, are under strict translational control.

Translation has an established role in cell growth. Basically, an increase in protein synthesis occurs as a consequence of mitogenesis. Until recently, however, little was known about the alterations in mRNA translation in cancer, and much is yet to be discovered about their role in the development and progression of cancer. Here we review the basic principles of translational control, the alterations encountered in cancer, and selected therapies targeting translation initiation to elucidate potential new therapeutic avenues.

Basic Principles of Translational Control

Mechanism of Translation Initiation

Translation Initiation is the main step in translational regulation. Translation initiation is a complex process in which the initiator tRNA and the 40S and 60S ribosomal subunits are recruited to the 5' end of a mRNA molecule and assembled by eukaryotic translation initiation factors into an 80S ribosome at the start codon of the mRNA (Fig. 1). The 5' end of eukaryotic mRNA is capped, *i.e.*, contains the cap structure m⁷GpppN (7-methylguanosine-triphospho-5'-ribonucleoside). Most translation in eukaryotes occurs in a cap-dependent fashion, *i.e.*, the cap is specifically recognized by the eIF4E,³ which binds the 5' cap. The eIF4F translation initiation complex is then formed by the assembly of eIF4E, the RNA helicase eIF4A, and eIF4G, a scaffolding protein that mediates the binding of the 40S ribosomal subunit to the mRNA molecule through interaction with the eIF3 protein present on the 40S ribosome. eIF4A and eIF4B participate in melting the secondary structure of the 5' UTR of the mRNA. The 43S initiation complex (40S/eIF2/Met-tRNA/GTP complex) scans the mRNA in a 5'→3' direction until it encounters an AUG start codon. This start codon is then base-paired to the anticodon of initiator tRNA, forming the 48S initiation complex. The initiation factors are then displaced from the 48S complex, and the 60S ribosome joins to form the 80S ribosome.

Unlike most eukaryotic translation, translation initiation of certain mRNAs, such as the picornavirus RNA, is cap independent and occurs by internal ribosome entry. This mechanism does not require eIF4E. Either the 43S complex can bind the initiation codon directly through interaction with the IRES in the 5' UTR such as in the encephalomyocarditis virus, or it can

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² To whom requests for reprints should be addressed, at Department of Surgical Oncology, Box 444, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 745-4453; Fax: (713) 745-4926; E-mail: fmeric@mdanderson.org.

³ The abbreviations used are: eIF4E, eukaryotic initiation factor 4E; UTR, untranslated region; IRES, internal ribosome entry site; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; S6K, ribosomal p70 S6 kinase; mTOR, mammalian target of rapamycin; ATM, ataxia telangiectasia mutated; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted from chromosome 10; PP2A, protein phosphatase 2A; TGF- β 3, transforming growth factor- β 3; PAP, poly(A) polymerase; EPA, eicosopentaenoic acid; mda-7, melanoma differentiation-associated gene 7.

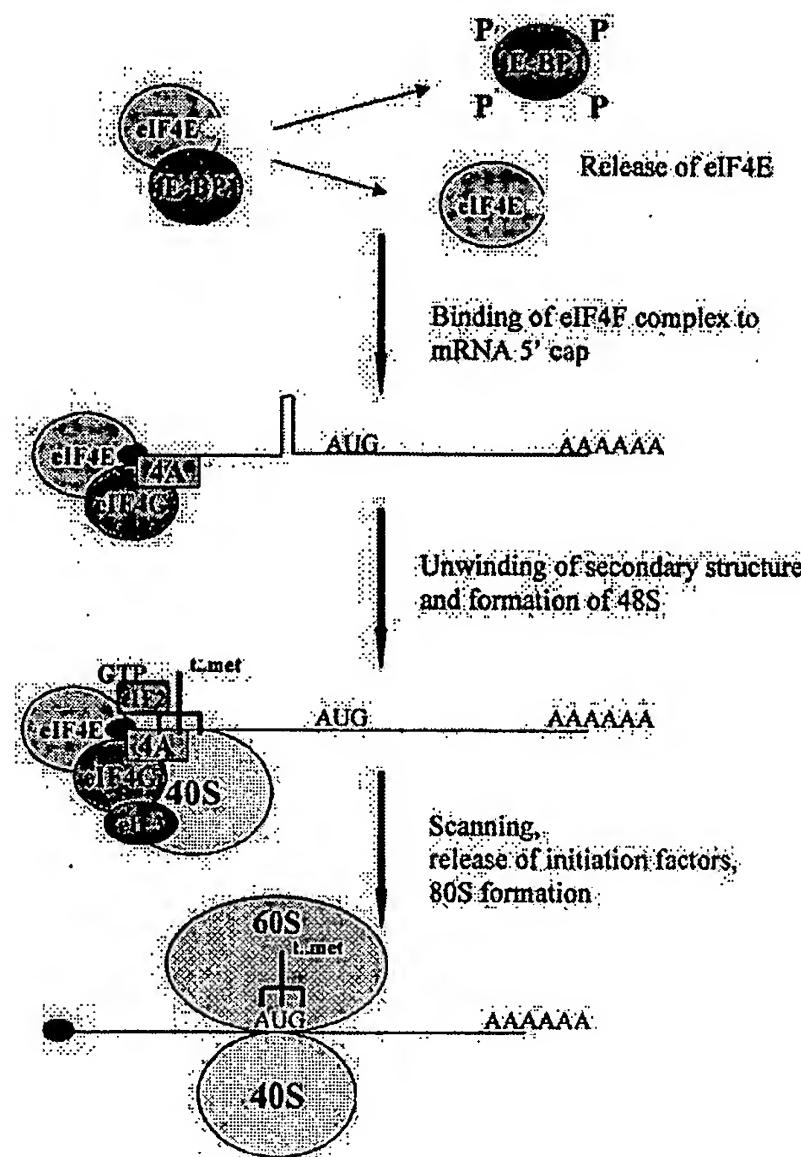


Fig. 1. Translation initiation in eukaryotes. The 4E-BPs are hyperphosphorylated to release eIF4E so that it can interact with the 5' cap, and the eIF4F initiation complex is assembled. The interaction of poly(A) binding protein with the initiation complex and circularization of the mRNA is not depicted in the diagram. The secondary structure of the 5' UTR is melted, the 40S ribosomal subunit is bound to eIF3, and the ternary complex consisting of eIF2, GTP, and the Met-tRNA are recruited to the mRNA. The ribosome scans the mRNA in a 5' → 3' direction until an AUG start codon is found in the appropriate sequence context. The initiation factors are released, and the large ribosomal subunit is recruited.

initially attach to the IRES and then reach the initiation codon by scanning or transfer, as is the case with the poliovirus (1).

Regulation of Translation Initiation

Translation initiation can be regulated by alterations in the expression or phosphorylation status of the various factors involved. Key components in translational regulation that may provide potential therapeutic targets follow.

eIF4E. eIF4E plays a central role in translation regulation. It is the least abundant of the initiation factors and is considered the rate-limiting component for initiation of cap-dependent translation. eIF4E may also be involved in mRNA splicing, mRNA 3' processing, and mRNA nucleocytoplasmic transport (2). eIF4E expression can be increased at the transcriptional level in response to serum or growth factors (3). eIF4E overexpression may cause preferential translation of mRNAs containing excessive secondary structure in their 5' UTR that are normally discriminated against by the trans-

lational machinery and thus are inefficiently translated (4–7). As examples of this, overexpression of eIF4E promotes increased translation of vascular endothelial growth factor, fibroblast growth factor-2, and cyclin D1 (2, 8, 9).

Another mechanism of control is the regulation of eIF4E phosphorylation. eIF4E phosphorylation is mediated by the mitogen-activated protein kinase-interacting kinase 1, which is activated by the mitogen-activated pathway activating extracellular signal-related kinases and the stress-activated pathway acting through p38 mitogen-activated protein kinase (10–13). Several mitogens, such as serum, platelet-derived growth factor, epidermal growth factor, insulin, angiotensin II, src kinase overexpression, and ras overexpression, lead to eIF4E phosphorylation (14). The phosphorylation status of eIF4E is usually correlated with the translational rate and growth status of the cell; however, eIF4E phosphorylation has also been observed in response to some cellular stresses when translational rates actually decrease (15). Thus, further study is needed to understand the effects of eIF4E phosphorylation on eIF4E activity.

Another mechanism of regulation is the alteration of eIF4E availability by the binding of eIF4E to the eIF4E-binding proteins (4E-BP, also known as PHAS-I). 4E-BPs compete with eIF4G for a binding site in eIF4E. The binding of eIF4E to the best characterized eIF4E-binding protein, 4E-BP1, is regulated by 4E-BP1 phosphorylation. Hypophosphorylated 4E-BP1 binds to eIF4E, whereas 4E-BP1 hyperphosphorylation decreases this binding. Insulin, angiotensin, epidermal growth factor, platelet-derived growth factor, hepatocyte growth factor, nerve growth factor, insulin-like growth factors I and II, interleukin 3, granulocyte-macrophage colony-stimulating factor + steel factor, gastrin, and the adenovirus have all been reported to induce phosphorylation of 4E-BP1 and to decrease the ability of 4E-BP1 to bind eIF4E (15, 16). Conversely, deprivation of nutrients or growth factors results in 4E-BP1 dephosphorylation, an increase in eIF4E binding, and a decrease in cap-dependent translation.

p70 S6 Kinase. Phosphorylation of ribosomal 40S protein S6 by S6K is thought to play an important role in translational regulation. S6K^{−/−} mouse embryonic cells proliferate more slowly than do parental cells, demonstrating that S6K has a positive influence on cell proliferation (17). S6K regulates the translation of a group of mRNAs possessing a 5' terminal oligopyrimidine tract (5' TOP) found at the 5' UTR of ribosomal protein mRNAs and other mRNAs coding for components of the translational machinery. Phosphorylation of S6K is regulated in part based on the availability of nutrients (18, 19) and is stimulated by several growth factors, such as platelet-derived growth factor and insulin-like growth factor I (20).

eIF2α Phosphorylation. The binding of the initiator tRNA to the small ribosomal unit is mediated by translation initiation factor eIF2. Phosphorylation of the α-subunit of eIF2 prevents formation of the eIF2/GTP/Met-tRNA complex and inhibits global protein synthesis (21, 22). eIF2α is phosphorylated under a variety of conditions, such as viral infection, nutrient deprivation, heme deprivation, and apoptosis (22). eIF2α is phosphorylated by heme-regulated inhibitor, nutrient-regulated protein kinase, and the IFN-induced, double-stranded RNA-activated protein kinase (PKR; Ref. 23).

The mTOR Signaling Pathway. The macrolide antibiotic rapamycin (Spiralimus; Wyeth-Ayerst Research, Collegeville, PA) has been the subject of intensive study because it inhibits signal transduction pathways involved in T-cell activation. The rapamycin-sensitive component of these pathways is mTOR (also called FRAP or RAFT1). mTOR is the mammalian homologue of the yeast TOR proteins that regulate G₁ progression and translation in response to nutrient availability (24). mTOR is a serine-threonine kinase that modulates translation initiation by altering the phosphorylation status of 4E-BP1 and S6K (Fig. 2; Ref. 25).

4E-BP1 is phosphorylated on multiple residues. mTOR phosphorylates the Thr-37 and Thr-46 residues of 4E-BP1 *in vitro* (26); however, phosphorylation at these sites is not associated with a loss of eIF4E binding. Phosphorylation of Thr-37 and Thr-46 is required for subsequent phosphorylation at several COOH-terminal, serum-sensitive sites; a combination of these phosphorylation events appears to be needed to inhibit the binding of 4E-BP1 to eIF4E (25). The product of the ATM gene, p38/MSK1 pathway, and protein kinase C σ also play a role in 4E-BP1 phosphorylation (27–29).

S6K and 4E-BP1 are also regulated, in part, by PI3K and its downstream protein kinase Akt. PTEN is a phosphatase that negatively regulates PI3K signaling. PTEN null cells have constitutively active Akt, with increased S6K activity and S6 phosphorylation (30). S6K activity is inhibited both by PI3K inhibitors wortmannin and LY294002 and by mTOR inhibitor rapamycin (24). Akt phosphorylates Ser-2448 in mTOR *in vitro*, and this site is phosphorylated upon Akt activation *in vivo* (31–33). Thus, mTOR is regulated by the PI3K/Akt pathway; however, this does not appear to be the only mode of regulation of mTOR activity. Whether the PI3K pathway also regulates S6K and 4E-BP1 phosphorylation independent of mTOR is controversial.

Interestingly, mTOR autophosphorylation is blocked by wortmannin but not by rapamycin (34). This seeming inconsistency suggests that mTOR-responsive regulation of 4E-BP1 and S6K activity occurs through a mechanism other than intrinsic mTOR kinase activity. An alternate pathway for 4E-BP1 and S6K phosphorylation by mTOR activity is by the inhibition of a phosphatase. Treatment with calyculin A, an inhibitor of phosphatases 1 and 2A, reduces rapamycin-induced dephosphorylation of 4E-BP1 and S6K by rapamycin (35). PP2A interacts with full-length S6K but not with a S6K mutant that is resistant to dephosphorylation resulting from rapamycin. mTOR phosphorylates PP2A *in vitro*; however, how this process alters PP2A activity is not known. These results are consistent with the model that phosphorylation of a phosphatase by mTOR prevents dephosphorylation of 4E-BP1 and S6K, and conversely, that nutrient deprivation and rapamycin block inhibition of the phosphatase by mTOR.

Polyadenylation. The poly(A) tail in eukaryotic mRNA is important in enhancing translation initiation and mRNA stability. Polyadenylation plays a key role in regulating gene expression during oogenesis and early embryogenesis. Some mRNA that are translationally inactive in the oocyte are polyadenylated concomitantly with translational activation in oocyte maturation, whereas other mRNAs that are translationally active during oogenesis are deadenylated and trans-

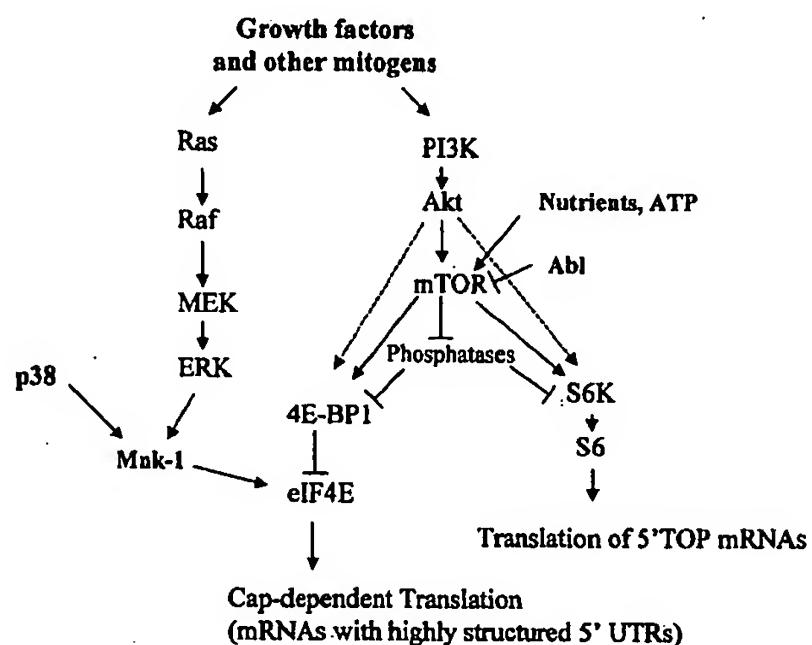


Fig. 2. Regulation of translation initiation by signal transduction pathways. Signaling via p38, extracellular signal-related kinase, PI3K, and mTOR can all activate translation initiation.

lationally silenced (36–38). Thus, control of poly(A) tail synthesis is an important regulatory step in gene expression. The 5' cap and poly(A) tail are thought to function synergistically to regulate mRNA translational efficiency (39, 40).

RNA Packaging. Most RNA-binding proteins are assembled on a transcript at the time of transcription, thus determining the translational fate of the transcript (41). A highly conserved family of Y-box proteins is found in cytoplasmic messenger ribonucleoprotein particles, where the proteins are thought to play a role in restricting the recruitment of mRNA to the translational machinery (41–43). The major mRNA-associated protein, YB-1, destabilizes the interaction of eIF4E and the 5' mRNA cap *in vitro*, and overexpression of YB-1 results in translational repression *in vivo* (44). Thus, alterations in RNA packaging can also play an important role in translational regulation.

Translation Alterations Encountered in Cancer

Three main alterations at the translational level occur in cancer: variations in mRNA sequences that increase or decrease translational efficiency, changes in the expression or availability of components of the translational machinery, and activation of translation through aberrantly activated signal transduction pathways. The first alteration affects the translation of an individual mRNA that may play a role in carcinogenesis. The second and third alterations can lead to more global changes, such as an increase in the overall rate of protein synthesis, and the translational activation of several mRNA species.

Variations in mRNA Sequence

Variations in mRNA sequence affect the translational efficiency of the transcript. A brief description of these variations and examples of each mechanism follow.

Mutations. Mutations in the mRNA sequence, especially in the 5' UTR, can alter its translational efficiency, as seen in the following examples.

c-myc. Saito *et al.* proposed that translation of full-length *c-myc* is repressed, whereas in several Burkitt lymphomas that have deletions of the mRNA 5' UTR, translation of *c-myc* is more efficient (45). More recently, it was reported that the 5' UTR of *c-myc* contains an IRES, and thus *c-myc* translation can be initiated by a cap-independent as well as a cap-dependent mechanism (46, 47). In patients with multiple myeloma, a C→T mutation in the *c-myc* IRES was identified (48) and found to cause an enhanced initiation of translation via internal ribosomal entry (49).

BRCA1. A somatic point mutation (117 G→C) in position -3 with respect to the start codon of the *BRCA1* gene was identified in a highly aggressive sporadic breast cancer (50). Chimeric constructs consisting of the wild-type or mutated *BRCA1* 5' UTR and a downstream luciferase reporter demonstrated a decrease in the translational efficiency with the 5' UTR mutation.

Cyclin-dependent Kinase Inhibitor 2A. Some inherited melanoma kindreds have a G→T transversion at base -34 of cyclin-dependent kinase inhibitor-2A, which encodes a cyclin-dependent kinase 4/cyclin-dependent kinase 6 kinase inhibitor important in G₁ checkpoint regulation (51). This mutation gives rise to a novel AUG translation initiation codon, creating an upstream open reading frame that competes for scanning ribosomes and decreases translation from the wild-type AUG.

Alternate Splicing and Alternate Transcription Start Sites. Alterations in splicing and alternate transcription sites can lead to variations in 5' UTR sequence, length, and secondary structure, ultimately impacting translational efficiency.

ATM. The *ATM* gene has four noncoding exons in its 5' UTR that undergo extensive alternative splicing (52). The contents of 12 different 5' UTRs that show considerable diversity in length and sequence have been identified. These divergent 5' leader sequences play an important role in the translational regulation of the *ATM* gene.

mdm. In a subset of tumors, overexpression of the oncoprotein *mdm2* results in enhanced translation of the *mdm2* mRNA. Use of different promoters leads to two *mdm2* transcripts that differ only in their 5' leaders (53). The longer 5' UTR contains two upstream open reading frames, and this mRNA is loaded with ribosomes inefficiently compared with the short 5' UTR.

BRCA1. In a normal mammary gland, *BRCA1* mRNA is expressed with a shorter leader sequence (5'UTRa), whereas in sporadic breast cancer tissue, *BRCA1* mRNA is expressed with a longer leader sequence (5' UTRb); the translational efficiency of transcripts containing 5' UTRb is 10 times lower than that of transcripts containing 5' UTRa (54).

TGF-β3. *TGF-β3* mRNA includes a 1.1-kb 5' UTR, which exerts an inhibitory effect on translation. Many human breast cancer cell lines contain a novel *TGF-β3* transcript with a 5' UTR that is 870 nucleotides shorter and has a 7-fold greater translational efficiency than the normal *TGF-β3* mRNA (55).

Alternate Polyadenylation Sites. Multiple polyadenylation signals leading to the generation of several transcripts with differing 3' UTR have been described for several mRNA species, such as the *RET* proto-oncogene (56), *ATM* gene (52), tissue inhibitor of metalloproteinases-3 (57), *RHOA*

proto-oncogene (58), and calmodulin-1 (59). Although the effect of these alternate 3' UTRs on translation is not yet known, they may be important in RNA-protein interactions that affect translational recruitment. The role of these alterations in cancer development and progression is unknown.

Alterations in the Components of the Translation Machinery

Alterations in the components of translation machinery can take many forms.

Overexpression of eIF4E. Overexpression of eIF4E causes malignant transformation in rodent cells (60) and the deregulation of HeLa cell growth (61). Polunovsky *et al.* (62) found that eIF4E overexpression substitutes for serum and individual growth factors in preserving viability of fibroblasts, which suggests that eIF4E can mediate both proliferative and survival signaling.

Elevated levels of eIF4E mRNA have been found in a broad spectrum of transformed cell lines (63). eIF4E levels are elevated in all ductal carcinoma *in situ* specimens and invasive ductal carcinomas, compared with benign breast specimens evaluated with Western blot analysis (64, 65). Preliminary studies suggest that this overexpression is attributable to gene amplification (66).

There are accumulating data suggesting that eIF4E overexpression can be valuable as a prognostic marker. eIF4E overexpression was found in a retrospective study to be a marker of poor prognosis in stages I to III breast carcinoma (67). Verification of the prognostic value of eIF4E in breast cancer is now under way in a prospective trial (67). However, in a different study, eIF4E expression was correlated with the aggressive behavior of non-Hodgkin's lymphomas (68). In a prospective analysis of patients with head and neck cancer, elevated levels of eIF4E in histologically tumor-free surgical margins predicted a significantly increased risk of local-regional recurrence (9). These results all suggest that eIF4E overexpression can be used to select patients who might benefit from more aggressive systemic therapy. Furthermore, the head and neck cancer data suggest that eIF4E overexpression is a field defect and can be used to guide local therapy.

Alterations In Other Initiation Factors. Alterations in a number of other initiation factors have been associated with cancer. Overproduction of eIF4G, similar to eIF4E, leads to malignant transformation *in vitro* (69). eIF-2α is found in increased levels in bronchioloalveolar carcinomas of the lung (3). Initiation factor eIF-4A1 is overexpressed in melanoma (70) and hepatocellular carcinoma (71). The p40 subunit of translation initiation factor 3 is amplified and overexpressed in breast and prostate cancer (72), and the eIF3-p110 subunit is overexpressed in testicular seminoma (73). The role that overexpression of these initiation factors plays on the development and progression of cancer, if any, is not known.

Overexpression of S6K. S6K is amplified and highly overexpressed in the MCF7 breast cancer cell line, compared with normal mammary epithelium (74). In a study by Barlund *et al.* (74), S6K was amplified in 59 of 668 primary breast tumors, and a statistically significant association was observed between amplification and poor prognosis.

Overexpression of PAP. PAP catalyzes 3' poly(A) synthesis. PAP is overexpressed in human cancer cells compared with normal and virally transformed cells (75). PAP enzymatic activity in breast tumors has been correlated with PAP protein levels (76) and, in mammary tumor cytosols, was found to be an independent factor for predicting survival (76). Little is known, however, about how PAP expression or activity affects the translational profile.

Alterations in RNA-binding Proteins. Even less is known about alterations in RNA packaging in cancer. Increased expression and nuclear localization of the RNA-binding protein YB-1 are indicators of a poor prognosis for breast cancer (77), non-small cell lung cancer (78), and ovarian cancer (79). However, this effect may be mediated at least in part at the level of transcription, because YB-1 increases chemoresistance by enhancing the transcription of a multidrug resistance gene (80).

Activation of Signal Transduction Pathways

Activation of signal transduction pathways by loss of tumor suppressor genes or overexpression of certain tyrosine kinases can contribute to the growth and aggressiveness of tumors. An important mutant in human cancers is the tumor suppressor gene *PTEN*, which leads to the activation of the PI3K/Akt pathway. Activation of PI3K and Akt induces the oncogenic transformation of chicken embryo fibroblasts. The transformed cells show constitutive phosphorylation of S6K and of 4E-BP1 (81). A mutant Akt that retains kinase activity but does not phosphorylate S6K or 4E-BP1 does not transform fibroblasts, which suggests a correlation between the oncogenicity of PI3K and Akt and the phosphorylation of S6K and 4E-BP1 (81).

Several tyrosine kinases such as platelet-derived growth factor, insulin-like growth factor, HER2/neu, and epidermal growth factor receptor are overexpressed in cancer. Because these kinases activate downstream signal transduction pathways known to alter translation initiation, activation of translation is likely to contribute to the growth and aggressiveness of these tumors. Furthermore, the mRNA for many of these kinases themselves are under translational control. For example, HER2/neu mRNA is translationally controlled both by a short upstream open reading frame that represses HER2/neu translation in a cell type-independent manner and by a distinct cell type-dependent mechanism that increases translational efficiency (82). HER2/neu translation is different in transformed and normal cells. Thus, it is possible that alterations at the translational level can in part account for the discrepancy between *HER2/neu* gene amplification detected by fluorescence *in situ* hybridization and protein levels detected by immunohistochemical assays.

Translation Targets of Selected Cancer Therapy

Components of the translation machinery and signal pathways involved in the activation of translation initiation represent good targets for cancer therapy.

Targeting the mTOR Signaling Pathway: Rapamycin and Tumstatin

Rapamycin inhibits the proliferation of lymphocytes. It was initially developed as an immunosuppressive drug for organ

transplantation. Rapamycin with FKBP 12 (FK506-binding protein, M_r , 12,000) binds to mTOR to inhibit its function.

Rapamycin causes a small but significant reduction in the initiation rate of protein synthesis (83). It blocks cell growth in part by blocking S6 phosphorylation and selectively suppressing the translation of 5' TOP mRNAs, such as ribosomal proteins, and elongation factors (83-85). Rapamycin also blocks 4E-BP1 phosphorylation and inhibits cap-dependent but not cap-independent translation (17, 86).

The rapamycin-sensitive signal transduction pathway, activated during malignant transformation and cancer progression, is now being studied as a target for cancer therapy (87). Prostate, breast, small cell lung, glioblastoma, melanoma, and T-cell leukemia are among the cancer lines most sensitive to the rapamycin analogue CCI-779 (Wyeth-Ayerst Research; Ref. 87). In rhabdomyosarcoma cell lines, rapamycin is either cytostatic or cytotoxic, depending on the p53 status of the cell; p53 wild-type cells treated with rapamycin arrest in the G₁ phase and maintain their viability, whereas p53 mutant cells accumulate in G₁ and undergo apoptosis (88, 89). In a recently reported study using human primitive neuroectodermal tumor and medulloblastoma models, rapamycin exhibited more cytotoxicity in combination with cisplatin and camptothecin than as a single agent. *In vivo*, CCI-779 delayed growth of xenografts by 160% after 1 week of therapy and 240% after 2 weeks. A single high-dose administration caused a 37% decrease in tumor volume. Growth inhibition *in vivo* was 1.3 times greater, with cisplatin in combination with CCI-779 than with cisplatin alone (90). Thus, preclinical studies suggest that rapamycin analogues are useful as single agents and in combination with chemotherapy.

Rapamycin analogues CCI-779 and RAD001 (Novartis, Basel, Switzerland) are now in clinical trials. Because of the known effect of rapamycin on lymphocyte proliferation, a potential problem with rapamycin analogues is immunosuppression. However, although prolonged immunosuppression can result from rapamycin and CCI-779 administered on continuous-dose schedules, the immunosuppressive effects of rapamycin analogues resolve in ~24 h after therapy (91). The principal toxicities of CCI-779 have included dermatological toxicity, myelosuppression, infection, mucositis, diarrhea, reversible elevations in liver function tests, hyperglycemia, hypokalemia, hypocalcemia, and depression (87, 92-94). Phase II trials of CCI-779 have been conducted in advanced renal cell carcinoma and in stage III/IV breast carcinoma patients who failed with prior chemotherapy. In the results reported in abstract form, although there were no complete responses, partial responses were documented in both renal cell carcinoma and in breast carcinoma (94, 95). Thus, CCI-779 has documented preliminary clinical activity in a previously treated, unselected patient population.

Active investigation is under way into patient selection for mTOR inhibitors. Several studies have found an enhanced efficacy of CCI-779 in PTEN-null tumors (30, 96). Another study found that six of eight breast cancer cell lines were responsive to CCI-779, although only two of these lines lacked PTEN (97). There was, however, a positive correlation between Akt activation and CCI-779 sensitivity (97). This correlation suggests that activation of the PI3K-Akt pathway,

regardless of whether it is attributable to a PTEN mutation or to overexpression of receptor tyrosine kinases, makes cancer cell amenable to mTOR-directed therapy. In contrast, lower levels of the target of mTOR, 4E-BP1, are associated with rapamycin resistance; thus, a lower 4E-BP1/eIF4E ratio may predict rapamycin resistance (98).

Another mode of activity for rapamycin and its analogues appears to be through inhibition of angiogenesis. This activity may be both through direct inhibition of endothelial cell proliferation as a result of mTOR inhibition in these cells or by inhibition of translation of such proangiogenic factors as vascular endothelial growth factor in tumor cells (99, 100).

The angiogenesis inhibitor tumstatin, another anticancer drug currently under study, was also found recently to inhibit translation in endothelial cells (101). Through a requisite interaction with integrin, tumstatin inhibits activation of the PI3K/Akt pathway and mTOR in endothelial cells and prevents dissociation of eIF4E from 4E-BP1, thereby inhibiting cap-dependent translation. These findings suggest that endothelial cells are especially sensitive to therapies targeting the mTOR-signaling pathway.

Targeting eIF2 α : EPA, Clotrimazole, mda-7, and Flavonoids

EPA is an n-3 polyunsaturated fatty acid found in the fish-based diets of populations having a low incidence of cancer (102). EPA inhibits the proliferation of cancer cells (103), as well as in animal models (104, 105). It blocks cell division by inhibiting translation initiation (105). EPA releases Ca^{2+} from intracellular stores while inhibiting their refilling, thereby activating PKR. PKR, in turn phosphorylates and inhibits eIF2 α , resulting in the inhibition of protein synthesis at the level of translation initiation. Similarly, clotrimazole, a potent antiproliferative agent *in vitro* and *in vivo*, inhibits cell growth through depletion of Ca^{2+} stores, activation of PKR, and phosphorylation of eIF2 α (106). Consequently, clotrimazole preferentially decreases the expression of cyclins A, E, and D1, resulting in blockage of the cell cycle in G₁.

mda-7 is a novel tumor suppressor gene being developed as a gene therapy agent. Adenoviral transfer of *mda-7* (Ad-*mda7*) induces apoptosis in many cancer cells including breast, colorectal, and lung cancer (107–109). Ad-*mda7* also induces and activates PKR, which leads to phosphorylation of eIF2 α and induction of apoptosis (110).

Flavonoids such as genistein and quercetin suppress tumor cell growth. All three mammalian eIF2 α kinases, PKR, heme-regulated inhibitor, and PERK/PEK, are activated by flavonoids, with phosphorylation of eIF2 α and inhibition of protein synthesis (111).

Targeting eIF4A and eIF4E: Antisense RNA and Peptides

Antisense expression of eIF4A decreases the proliferation rate of melanoma cells (112). Sequestration of eIF4E by overexpression of 4E-BP1 is proapoptotic and decreases tumorigenicity (113, 114). Reduction of eIF4E with antisense RNA decreases soft agar growth, increases tumor latency, and increases the rates of tumor doubling times (7). Antisense eIF4E RNA treat-

ment also reduces the expression of angiogenic factors (115) and has been proposed as a potential adjuvant therapy for head and neck cancers, particularly when elevated eIF4E is found in surgical margins. Small molecule inhibitors that bind the eIF4G/4E-BP1-binding domain of eIF4E are proapoptotic (116) and are also being actively pursued.

Exploiting Selective Translation for Gene Therapy

A different therapeutic approach that takes advantage of the enhanced cap-dependent translation in cancer cells is the use of gene therapy vectors encoding suicide genes with highly structured 5' UTR. These mRNA would thus be at a competitive disadvantage in normal cells and not translate well, whereas in cancer cells, they would translate more efficiently. For example, the introduction of the 5' UTR of fibroblast growth factor-2 5' to the coding sequence of *herpes simplex virus type-1 thymidine kinase* gene, allows for selective translation of *herpes simplex virus type-1 thymidine kinase* gene in breast cancer cell lines compared with normal mammary cell lines and results in selective sensitivity to ganciclovir (117).

Toward the Future

Translation is a crucial process in every cell. However, several alterations in translational control occur in cancer. Cancer cells appear to need an aberrantly activated translational state for survival, thus allowing the targeting of translation initiation with surprisingly low toxicity. Components of the translational machinery, such as eIF4E, and signal transduction pathways involved in translation initiation, such mTOR, represent promising targets for cancer therapy. Inhibitors of the mTOR have already shown some preliminary activity in clinical trials. It is possible that with the development of better predictive markers and better patient selection, response rates to single-agent therapy can be improved. Similar to other cytostatic agents, however, mTOR inhibitors are most likely to achieve clinical utility in combination therapy. In the interim, our increasing understanding of translation initiation and signal transduction pathways promise to lead to the identification of new therapeutic targets in the near future.

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References

1. Pestova, T. V., Kolupaeva, V. G., Lomakin, I. B., Pilipenko, E. V., Shatsky, I. N., Agol, V. I., and Hellen, C. U. Molecular mechanisms of translation initiation in eukaryotes. *Proc. Natl. Acad. Sci. USA*, 98: 7029–7036, 2001.
2. Rosenwald, I. B., Kaspar, R., Rousseau, D., Gehrke, L., Leboulch, P., Chen, J. J., Schmidt, E. V., Sonenberg, N., and London, I. M. Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels. *J. Biol. Chem.*, 270: 21176–21180, 1995.
3. Rosenwald, I. B., Hutzler, M. J., Wang, S., Savas, L., and Fraire, A. E. Expression of eukaryotic translation initiation factors 4E and 2 α is increased frequently in bronchioloalveolar but not in squamous cell carcinomas of the lung. *Cancer (Phila.)*, 92: 2164–2171, 2001.

4. Darveau, A., Pelletier, J., and Sonenberg, N. Differential efficiencies of *in vitro* translation of mouse c-myc transcripts differing in the 5' untranslated region. *Proc. Natl. Acad. Sci. USA*, 82: 2315-2319, 1985.
5. Kozak, M. Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. *Proc. Natl. Acad. Sci. USA*, 83: 2850-2854, 1986.
6. Koromilas, A. E., Lazaris-Karatzas, A., and Sonenberg, N. mRNAs containing extensive secondary structure in their 5' non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *EMBO J.*, 11: 4153-4158, 1992.
7. Rinker-Schaeffer, C. W., Graff, J. R., De Benedetti, A., Zimmer, S. G., and Rhoads, R. E. Decreasing the level of translation initiation factor 4E with antisense RNA causes reversal of ras-mediated transformation and tumorigenesis of cloned rat embryo fibroblasts. *Int. J. Cancer*, 55: 841-847, 1993.
8. Kevil, C. G., De Benedetti, A., Payne, D. K., Coe, L. L., Laroux, F. S., and Alexander, J. S. Translational regulation of vascular permeability factor by eukaryotic initiation factor 4E: implications for tumor angiogenesis. *Int. J. Cancer*, 65: 785-790, 1996.
9. Nathan, C. A., Franklin, S., Abreo, F. W., Nassar, R., De Benedetti, A., and Glass, J. Analysis of surgical margins with the molecular marker eIF4E: a prognostic factor in patients with head and neck cancer. *J. Clin. Oncol.*, 17: 2909-2914, 1999.
10. Fukunaga, R., and Hunter, T. MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.*, 16: 1921-1933, 1997.
11. Waskiewicz, A. J., Flynn, A., Proud, C. G., and Cooper, J. A. Mitogen-activated protein kinases activate the serine/threonine kinases MnK1 and MnK2. *EMBO J.*, 16: 1909-1920, 1997.
12. Wang, X., Flynn, A., Waskiewicz, A. J., Webb, B. L., Vries, R. G., Baines, I. A., Cooper, J. A., and Proud, C. G. The phosphorylation of eukaryotic initiation factor eIF4E in response to phorbol esters, cell stresses, and cytokines is mediated by distinct MAP kinase pathways. *J. Biol. Chem.*, 273: 9373-9377, 1998.
13. Pyronnet, S., Imataka, H., Gingras, A. C., Fukunaga, R., Hunter, T., and Sonenberg, N. Human eukaryotic translation initiation factor 4G (eIF4G) recruits MnK1 to phosphorylate eIF4E. *EMBO J.*, 18: 270-279, 1999.
14. Kleijn, M., Schepers, G. C., Voorma, H. O., and Thomas, A. A. Regulation of translation initiation factors by signal transduction. *Eur. J. Biochem.*, 253: 531-544, 1998.
15. Raught, B., and Gingras, A. C. eIF4E activity is regulated at multiple levels. *Int. J. Biochem. Cell Biol.*, 31: 43-57, 1999.
16. Takeuchi, K., Shibamoto, S., Nagamine, K., Shigemori, I., Omura, S., Kitamura, N., and Ito, F. Signaling pathways leading to transcription and translation cooperatively regulate the transient increase in expression of c-Fos protein. *J. Biol. Chem.*, 276: 26077-26083, 2001.
17. Kawasome, H., Papst, P., Webb, S., Keller, G. M., Johnson, G. L., Gelfand, E. W., and Terada, N. Targeted disruption of p70(S6K) defines its role in protein synthesis and rapamycin sensitivity. *Proc. Natl. Acad. Sci. USA*, 95: 5033-5038, 1998.
18. Christie, G. R., Hajduch, E., Hundal, H. S., Proud, C. G., and Taylor, P. M. Intracellular sensing of amino acids in *Xenopus laevis* oocytes stimulates p70 S6 kinase in a target of rapamycin-dependent manner. *J. Biol. Chem.*, 277: 9952-9957, 2002.
19. Hara, K., Yonezawa, K., Weng, Q. P., Kozlowski, M. T., Belham, C., and Avruch, J. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J. Biol. Chem.*, 273: 14484-14494, 1998.
20. Graves, L. M., Bornfeldt, K. E., Argast, G. M., Krebs, E. G., Kong, X., Lin, T. A., and Lawrence, J. C., Jr. cAMP- and rapamycin-sensitive regulation of the association of eukaryotic initiation factor 4E and the translational regulator PHAS-I in aortic smooth muscle cells. *Proc. Natl. Acad. Sci. USA*, 92: 7222-7226, 1995.
21. Merrick, W. C., and Hershey, J. W. B. The pathway and mechanism of eukaryotic protein synthesis. In: J. W. B. Hershey and M. B. Mathews (eds.), *Translational Control*, pp. 31-69. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1996.
22. Kimball, S. R. Eukaryotic initiation factor eIF2. *Int. J. Biochem. Cell Biol.*, 31: 25-29, 1999.
23. Jagus, R., Joshi, B., and Barber, G. N. PKR, apoptosis and cancer. *Int. J. Biochem. Cell Biol.*, 31: 123-138, 1999.
24. Thomas, G., and Hall, M. N. TOR signalling and control of cell growth. *Curr. Opin. Cell Biol.*, 9: 782-787, 1997.
25. Gingras, A. C., Raught, B., and Sonenberg, N. Regulation of translation initiation by FRAP/mTOR. *Genes Dev.*, 15: 807-826, 2001.
26. Gingras, A. C., Gygi, S. P., Raught, B., Polakiewicz, R. D., Abraham, R. T., Hoekstra, M. F., Aebersold, R., and Sonenberg, N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev.*, 13: 1422-1437, 1999.
27. Kumar, V., Pandey, P., Sabatini, D., Kumar, M., Majumder, P. K., Bharti, A., Carmichael, G., Kufe, D., and Karbhandia, S. Functional interaction between RAFT1/FRAP/mTOR and protein kinase C δ in the regulation of cap-dependent initiation of translation. *EMBO J.*, 19: 1087-1097, 2000.
28. Yang, D. Q., and Kastan, M. B. Participation of ATM in insulin signalling through phosphorylation of eIF-4E-binding protein 1. *Nat. Cell. Biol.*, 2: 893-898, 2000.
29. Liu, G., Zhang, Y., Bode, A. M., Ma, W. Y., and Dong, Z. Phosphorylation of 4E-BP1 is mediated by the p38/MSK1 pathway in response to UVB irradiation. *J. Biol. Chem.*, 277: 8810-8816, 2002.
30. Neshat, M. S., Mellinghoff, I. K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J. J., Wu, H., and Sawyers, C. L. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc. Natl. Acad. Sci. USA*, 98: 10314-10319, 2001.
31. Sekulic, A., Hudson, C. C., Homme, J. L., Yin, P., Ottemess, D. M., Karnitz, L. M., and Abraham, R. T. A direct linkage between the phosphoinositide 3-kinase-AKT signalling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res.*, 60: 3504-3513, 2000.
32. Scott, P. H., and Lawrence, J. C., Jr. Attenuation of mammalian target of rapamycin activity by increased cAMP in 3T3-L1 adipocytes. *J. Biol. Chem.*, 273: 34496-34501, 1998.
33. Reynolds, I. T., Bodine, S. C., and Lawrence, J. C., Jr. Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. *J. Biol. Chem.*, 277: 17657-17662, 2002.
34. Peterson, R. T., Beal, P. A., Comb, M. J., and Schreiber, S. L. FKBP12-rapamycin-associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. *J. Biol. Chem.*, 275: 7416-7423, 2000.
35. Peterson, R. T., Desai, B. N., Hardwick, J. S., and Schreiber, S. L. Protein phosphatase 2A interacts with the 70-kDa S6 kinase and is activated by inhibition of FKBP12-rapamycin-associated protein. *Proc. Natl. Acad. Sci. USA*, 96: 4438-4442, 1999.
36. McGrew, L. L., Dworkin-Rastl, E., Dworkin, M. B., and Richter, J. D. Poly(A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element. *Genes Dev.*, 3: 803-815, 1989.
37. Sheets, M. D., Wu, M., and Wickens, M. Polyadenylation of c-mos mRNA as a control point in *Xenopus* meiotic maturation. *Nature (Lond.)*, 374: 511-516, 1995.
38. Varnum, S. M., and Wormington, W. M. Deadenylation of maternal mRNAs during *Xenopus* oocyte maturation does not require specific *cis*-sequences: a default mechanism for translational control. *Genes Dev.*, 4: 2278-2286, 1990.
39. Gallie, D. R. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev.*, 5: 2108-2116, 1991.
40. Sachs, A. B., and Varani, G. Eukaryotic translation initiation: there are (at least) two sides to every story. *Nat. Struct. Biol.*, 7: 356-361, 2000.
41. Wolffe, A. P., and Meric, F. Coupling transcription to translation: a novel site for the regulation of eukaryotic gene expression. *Int. J. Biochem. Cell Biol.*, 28: 247-257, 1996.
42. Evdokimova, V. M., Wei, C. L., Sitikov, A. S., Simonenko, P. N., Lazarev, O. A., Vasilenko, K. S., Ustinov, V. A., Hershey, J. W., and Ovchinnikov, L. P. The major protein of messenger ribonucleoprotein particles in somatic cells is a member of the Y-box binding transcription factor family. *J. Biol. Chem.*, 270: 3186-3192, 1995.
43. Matsumoto, K., Meric, F., and Wolffe, A. P. Translational repression dependent on the interaction of the *Xenopus* Y-box protein FRGY2 with mRNA. Role of the cold shock domain, tail domain, and selective RNA sequence recognition. *J. Biol. Chem.*, 271: 22706-22712, 1996.

44. Evdokimova, V., Ruzanov, P., Imataka, H., Raught, B., Svitkin, Y., Ovchinnikov, L. P., and Sonenberg, N. The major mRNA-associated protein YB-1 is a potent 5' cap-dependent mRNA stabilizer. *EMBO J.*, 20: 5491–5502, 2001.

45. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S., and Tonegawa, S. Activation of the *c-myc* gene by translocation: a model for translational control. *Proc. Natl. Acad. Sci. USA*, 80: 7476–7480, 1983.

46. Nanbru, C., Lafon, I., Audigier, S., Gensac, M. C., Vagner, S., Huez, G., and Prats, A. C. Alternative translation of the proto-oncogene *c-myc* by an internal ribosome entry site. *J. Biol. Chem.*, 272: 32061–32066, 1997.

47. Stoneley, M., Paulin, F. E., Le Quesne, J. P., Chappell, S. A., and Willis, A. E. *c-Myc* 5' untranslated region contains an internal ribosome entry segment. *Oncogene*, 16: 423–428, 1998.

48. Paulin, F. E., West, M. J., Sullivan, N. F., Whitney, R. L., Lyne, L., and Willis, A. E. Aberrant translational control of the *c-myc* gene in multiple myeloma. *Oncogene*, 13: 505–513, 1996.

49. Chappell, S. A., LeQuesne, J. P., Paulin, F. E., de Schoolmeester, M. L., Stoneley, M., Soutar, R. L., Ralston, S. H., Helfrich, M. H., and Willis, A. E. A mutation in the *c-myc*-IRES leads to enhanced internal ribosome entry in multiple myeloma: a novel mechanism of oncogene de-regulation. *Oncogene*, 19: 4437–4440, 2000.

50. Signori, E., Bagni, C., Papa, S., Primerano, B., Rinaldi, M., Amaldi, F., and Fazio, V. M. A. A somatic mutation in the 5'UTR of *BRCA1* gene in sporadic breast cancer causes down-modulation of translation efficiency. *Oncogene*, 20: 4596–4600, 2001.

51. Liu, L., Dilworth, D., Gao, L., Monzon, J., Summers, A., Lassam, N., and Hogg, D. Mutation of the *CDKN2A* 5' UTR creates an aberrant initiation codon and predisposes to melanoma. *Nat. Genet.*, 21: 128–132, 1999.

52. Savitsky, K., Platzer, M., Uziel, T., Gilad, S., Sartiel, A., Rosenthal, A., Elroy-Stein, O., Shiloh, Y., and Rotman, G. Ataxia-telangiectasia: structural diversity of untranslated sequences suggests complex post-transcriptional regulation of *ATM* gene expression. *Nucleic Acids Res.*, 25: 1678–1684, 1997.

53. Brown, C. Y., Mize, G. J., Pineda, M., George, D. L., and Morris, D. R. Role of two upstream open reading frames in the translational control of oncogene *mdm2*. *Oncogene*, 18: 5631–5637, 1999.

54. Sobczak, K., and Krzyzosiak, W. J. Structural determinants of *BRCA1* translational regulation. *J. Biol. Chem.*, 277: 17349–17358, 2002.

55. Arrick, B. A., Grendell, R. L., and Griffin, L. A. Enhanced translational efficiency of a novel transforming growth factor β 3 mRNA in human breast cancer cells. *Mol. Cell. Biol.*, 14: 619–628, 1994.

56. Myers, S. M., Eng, C., Ponder, B. A., and Mulligan, L. M. Characterization of *RET* proto-oncogene 3' splicing variants and polyadenylation sites: a novel C-terminus for *RET*. *Oncogene*, 11: 2039–2045, 1995.

57. Byrne, J. A., Tomasetto, C., Rouyer, N., Bellocq, J. P., Rio, M. C., and Basset, P. The tissue inhibitor of metalloproteinases-3 gene in breast carcinoma: identification of multiple polyadenylation sites and a stromal pattern of expression. *Mol. Med.*, 1: 418–427, 1995.

58. Moscow, J. A., He, R., Gudas, J. M., and Cowan, K. H. Utilization of multiple polyadenylation signals in the human *RHOA* protooncogene. *Gene (Amst.)*, 144: 229–236, 1994.

59. Senterre-Lesenfants, S., Alag, A. S., and Sobel, M. E. Multiple mRNA species are generated by alternate polyadenylation from the human *calmodulin-1* gene. *J. Cell. Biochem.*, 58: 445–454, 1995.

60. Lazaris-Karatzas, A., Montine, K. S., and Sonenberg, N. Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5' cap. *Nature (Lond.)*, 345: 544–547, 1990.

61. De Benedetti, A., and Rhoads, R. E. Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. *Proc. Natl. Acad. Sci. USA*, 87: 8212–8216, 1990.

62. Polunovsky, V. A., Rosenwald, I. B., Tan, A. T., White, J., Chiang, L., Sonenberg, N., and Bitterman, P. B. Translational control of programmed cell death: eukaryotic translation initiation factor 4E blocks apoptosis in growth-factor-restricted fibroblasts with physiologically expressed or deregulated Myc. *Mol. Cell. Biol.*, 16: 6573–6581, 1996.

63. Miyagi, Y., Sugiyama, A., Asai, A., Okazaki, T., Kuchino, Y., and Kerr, S. J. Elevated levels of eukaryotic translation initiation factor eIF-4E, mRNA in a broad spectrum of transformed cell lines. *Cancer Lett.*, 91: 247–252, 1995.

64. Kerekatte, V., Smiley, K., Hu, B., Smith, A., Gelder, F., and De Benedetti, A. The proto-oncogene/translation factor eIF4E: a survey of its expression in breast carcinomas. *Int. J. Cancer*, 64: 27–31, 1995.

65. Li, B. D., Liu, L., Dawson, M., and De Benedetti, A. Overexpression of eukaryotic initiation factor 4E (eIF4E) in breast carcinoma. *Cancer (Phila.)*, 79: 2385–2390, 1997.

66. Sorrells, D. L., Black, D. R., Meschonat, C., Rhoads, R., De Benedetti, A., Gao, M., Williams, B. J., and Li, B. D. Detection of eIF4E gene amplification in breast cancer by competitive PCR. *Ann. Surg. Oncol.*, 5: 232–237, 1998.

67. Li, B. D., McDonald, J. C., Nassar, R., and De Benedetti, A. Clinical outcome in stage I to III breast carcinoma and eIF4E overexpression. *Ann. Surg.*, 227: 756–761; discussion, 761–763, 1998.

68. Wang, S., Rosenwald, I. B., Hutzler, M. J., Pihan, G. A., Savas, L., Chen, J. J., and Woda, B. A. Expression of the eukaryotic translation initiation factors 4E and 2 α in non-Hodgkin's lymphomas. *Am. J. Pathol.*, 155: 247–255, 1999.

69. Fukuchi-Shimogori, T., Ishii, I., Kashiwagi, K., Mashiba, H., Ekimoto, H., and Igarashi, K. Malignant transformation by overproduction of translation initiation factor eIF4G. *Cancer Res.*, 57: 5041–5044, 1997.

70. Eberle, J., Krasagakis, K., and Orfanos, C. E. Translation initiation factor eIF-4A1 mRNA is consistently overexpressed in human melanoma cells *in vitro*. *Int. J. Cancer*, 71: 396–401, 1997.

71. Shuda, M., Kondoh, N., Tanaka, K., Ryo, A., Wakatsuki, T., Hada, A., Goseki, N., Igari, T., Hatsuse, K., Aihara, T., Horiuchi, S., Shichita, M., Yamamoto, N., and Yamamoto, M. Enhanced expression of translation factor mRNAs in hepatocellular carcinoma. *Anticancer Res.*, 20: 2489–2494, 2000.

72. Nupponen, N. N., Porkka, K., Kakkola, L., Tanner, M., Persson, K., Borg, A., Isola, J., and Visakorpi, T. Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer. *Am. J. Pathol.*, 154: 1777–1783, 1999.

73. Rothe, M., Ko, Y., Albers, P., and Wernert, N. Eukaryotic initiation factor 3 p110 mRNA is overexpressed in testicular seminomas. *Am. J. Pathol.*, 157: 1597–1604, 2000.

74. Barlund, M., Forozan, F., Kononen, J., Bubendorf, L., Chen, Y., Bittner, M. L., Torhorst, J., Haas, P., Bucher, C., Sauter, G., Kallioniemi, O. P., and Kallioniemi, A. Detecting activation of ribosomal protein S6 kinase by complementary DNA and tissue microarray analysis. *J. Natl. Cancer Inst. (Bethesda)*, 92: 1252–1259, 2000.

75. Topalian, S. L., Kaneko, S., Gonzales, M. I., Bond, G. L., Ward, Y., and Manley, J. L. Identification and functional characterization of neo-poly(A) polymerase, an RNA processing enzyme overexpressed in human tumors. *Mol. Cell. Biol.*, 21: 5614–5623, 2001.

76. Scorilas, A., Talieri, M., Ardagavanis, A., Courtis, N., Dimitriadis, E., Yotis, J., Tsiparis, C. M., and Trangas, T. Polyadenylate polymerase enzymatic activity in mammary tumor cytosols: a new independent prognostic marker in primary breast cancer. *Cancer Res.*, 60: 5427–5433, 2000.

77. Janz, M., Harbeck, N., Dettmar, P., Berger, U., Schmidt, A., Jurchott, K., Schmitt, M., and Royer, H. D. Y-box factor YB-1 predicts drug resistance and patient outcome in breast cancer independent of clinically relevant tumor biologic factors HER2, uPA and PAI-1. *Int. J. Cancer*, 97: 278–282, 2002.

78. Shibahara, K., Sugio, K., Osaki, T., Uchiumi, T., Maehara, Y., Kohno, K., Yasumoto, K., Sugimachi, K., and Kuwano, M. Nuclear expression of the Y-box binding protein, YB-1, as a novel marker of disease progression in non-small cell lung cancer. *Clin. Cancer Res.*, 7: 3151–3155, 2001.

79. Kamura, T., Yahata, H., Amada, S., Ogawa, S., Sonoda, T., Kobayashi, H., Mitsumoto, M., Kohno, K., Kuwano, M., and Nakano, H. Is nuclear expression of Y box-binding protein-1 a new prognostic factor in ovarian serous adenocarcinoma? *Cancer (Phila.)*, 85: 2450–2454, 1999.

80. Bargou, R. C., Jurchott, K., Wagener, C., Bergmann, S., Metzner, S., Bommert, K., Mapara, M. Y., Winzer, K. J., Dietel, M., Dorken, B., and Royer, H. D. Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic *MDR1* gene expression. *Nat. Med.*, 3: 447–450, 1997.

81. Aoki, M., Blazek, E., and Vogt, P. K. A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. *Proc. Natl. Acad. Sci. USA*, 98: 136–141, 2001.

82. Child, S. J., Miller, M. K., and Geballe, A. P. Cell type-dependent and -independent control of HER-2/neu translation. *Int. J. Biochem. Cell Biol.*, 31: 201–213, 1999.

83. Jefferies, H. B., Reinhard, C., Kozma, S. C., and Thomas, G. Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. *Proc. Natl. Acad. Sci. USA*, **91**: 4441–4445, 1994.

84. Terada, N., Patel, H. R., Takase, K., Kohno, K., Nairn, A. C., and Gelfand, E. W. Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. *Proc. Natl. Acad. Sci. USA*, **91**: 11477–11481, 1994.

85. Jefferies, H. B., Fumagalli, S., Dennis, P. B., Reinhard, C., Pearson, R. B., and Thomas, G. Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. *EMBO J.*, **16**: 3693–3704, 1997.

86. Beretta, L., Gingras, A. C., Svitkin, Y. V., Hall, M. N., and Sonenberg, N. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J.*, **15**: 658–664, 1996.

87. Hidalgo, M., and Rowinsky, E. K. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene*, **19**: 6680–6686, 2000.

88. Hosoi, H., Dilling, M. B., Shikata, T., Liu, L. N., Shu, L., Ashmun, R. A., Germain, G. S., Abraham, R. T., and Houghton, P. J. Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. *Cancer Res.*, **59**: 886–894, 1999.

89. Huang, S., and Houghton, P. J. Resistance to rapamycin: a novel anticancer drug. *Cancer Metastasis Rev.*, **20**: 69–78, 2001.

90. Georger, B., Kerr, K., Tang, C. B., Fung, K. M., Powell, B., Sutton, L. N., Phillips, P. C., and Janss, A. J. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res.*, **61**: 1527–1532, 2001.

91. Gibbons, J. J., Discafani, C., Peterson, R., Hernandez, R., Skotnicki, J., and Frost, P. The effect of CCI-779, a novel macrolide anti-tumor agent, on the growth of human tumor cells *in vitro* and in nude mouse xenografts *in vivo*. *Proc. Am. Assoc. Cancer Res.*, **40**: 301, 1999.

92. Hidalgo, M., Rowinsky, E., Erlichman, C., Marshall, B., Marks, R., Edwards, T., and Buckner, J. J. A Phase I and pharmacological study of CCI-779 cycle inhibitor. *Ann. Oncol.*, **11** (Suppl. 4): 133, 2001.

93. Alexandre, J., Raymond, E., Depenbrock, H., Mekhaldi, S., Angevin, E., Paillet, C., Hanauske, A., Frisch, J., Feussner, A., and Armand, J. P. CCI-779, a new rapamycin analog, has antitumor activity at doses inducing only mild cutaneous effects and mucositis: early results of an ongoing Phase I study. *Proceedings of the 1999 AACR-NCI-EORTC International Conference, Clin. Cancer Res.*, **5** (Suppl.): 3730s, 1999.

94. Chan, S., Johnston, S., Scheulen, M. E., Mross, K., Morant, A., Lahr, A., Feussner, A., Berger, M., and Kirsch, T. First report: a Phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. *Proc. Am. Soc. Clin. Oncol.*, **21**: 44a, 2002.

95. Atkins, M. B., Hidalgo, M., Stadler, W., Logan, T., Dutcher, J. P., Hudes, G., Park, Y., Marshall, B., Boni, J., and Dukart, G. A randomized double-blind Phase 2 study of intravenous CCI-779 administered weekly to patients with advanced renal cell carcinoma. *Proc. Am. Soc. Clin. Oncol.*, **21**: 10a, 2002.

96. Smith, S. G., Trinh, C. M., Inge, L. J., Thomas, G., Cloughsey, T. F., Sawyers, C. L., and Mischel, P. S. PTEN expression status predicts glioblastoma cell sensitivity to CCI-779. *Proc. Am. Assoc. Cancer Res.*, **43**: 335, 2002.

97. Yu, K., Toral-Barza, L., Discafani, C., Zhang, W. G., Skotnicki, J., Frost, P., and Gibbons, J. J. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr. Relat. Cancer*, **8**: 249–258, 2001.

98. Dilling, M. B., Germain, G. S., Dudkin, L., Jayaraman, A. L., Zhang, X., Harwood, F. C., and Houghton, P. J. 4E-binding proteins, the suppressors of eukaryotic initiation factor 4E, are downregulated in cells with acquired or intrinsic resistance to rapamycin. *J. Biol. Chem.*, **277**: 13907–13917, 2002.

99. Guba, M., von Breitenbuch, P., Steinbauer, M., Koehl, G., Flegel, S., Hornung, M., Bruns, C. J., Zuelke, C., Farkas, S., Anthuber, M., Jauch, K. W., and Geissler, E. K. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat. Med.*, **8**: 128–135, 2002.

100. Lane, H. A., Schell, C., Theuer, A., O'Reilly, T., and Wood, J. Antiangiogenic activity of RAD001, an orally active anticancer agent. *Proc. Am. Assoc. Cancer Res.*, **43**: 184, 2002.

101. Maeshima, Y., Sudhakar, A., Lively, J. C., Ueki, K., Kharbanda, S., Kahn, C. R., Sonenberg, N., Hynes, R. O., and Kalluri, R. Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. *Science (Wash. DC)*, **295**: 140–143, 2002.

102. Caygill, C. P., Charlett, A., and Hill, M. J. Fat, fish, fish oil and cancer. *Br. J. Cancer*, **74**: 159–164, 1996.

103. Falconer, J. S., Ross, J. A., Fearon, K. C., Hawkins, R. A., O'Riordain, M. G., and Carter, D. C. Effect of eicosapentaenoic acid and other fatty acids on the growth *in vitro* of human pancreatic cancer cell lines. *Br. J. Cancer*, **69**: 826–832, 1994.

104. Noguchi, M., Minami, M., Yagasaki, R., Kinoshita, K., Earashi, M., Kitagawa, H., Taniya, T., and Miyazaki, I. Chemoprevention of DMBA-induced mammary carcinogenesis in rats by low-dose EPA and DHA. *Br. J. Cancer*, **75**: 348–353, 1997.

105. Palakurthi, S. S., Fluckiger, R., Aktas, H., Changolkar, A. K., Shahsafaei, A., Harneit, S., Kilic, E., and Halperin, J. A. Inhibition of translation initiation mediates the anticancer effect of the n-3 polyunsaturated fatty acid eicosapentaenoic acid. *Cancer Res.*, **60**: 2919–2925, 2000.

106. Aktas, H., Fluckiger, R., Acosta, J. A., Savage, J. M., Palakurthi, S. S., and Halperin, J. A. Depletion of intracellular Ca^{2+} stores, phosphorylation of eIF2 α , and sustained inhibition of translation initiation mediate the anticancer effects of clotrimazole. *Proc. Natl. Acad. Sci. USA*, **95**: 8280–8285, 1998.

107. Mhashilkar, A. M., Schrock, R. D., Hindi, M., Liao, J., Sieger, K., Kourouma, F., Zou-Yang, X. H., Onishi, E., Takh, O., Vedick, T. S., Fanger, G., Stewart, L., Watson, G. J., Snary, D., Fisher, P. B., Saeki, T., Roth, J. A., Ramesh, R., and Chada, S. Melanoma differentiation associated gene-7 (*mda-7*): a novel anti-tumor gene for cancer gene therapy. *Mol. Med.*, **7**: 271–282, 2001.

108. Su, Z. Z., Madireddi, M. T., Lin, J. J., Young, C. S., Kitada, S., Reed, J. C., Goldstein, N. I., and Fisher, P. B. The cancer growth suppressor gene *mda-7* selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice. *Proc. Natl. Acad. Sci. USA*, **95**: 14400–14405, 1998.

109. Saeki, T., Mhashilkar, A., Chada, S., Branch, C., Roth, J. A., and Ramesh, R. Tumor-suppressive effects by adenovirus-mediated *mda-7* gene transfer in non-small cell lung cancer cell *in vitro*. *Gene Ther.*, **7**: 2051–2057, 2000.

110. Pataer, A., Vorburger, S. A., Barber, G. N., Chada, S., Mhashilkar, A. M., Zou-Yang, H., Stewart, A. L., Balachandran, S., Roth, J. A., Hunt, K. K., and Swisher, S. G. Adenoviral transfer of the melanoma differentiation-associated gene 7 (*mda7*) induces apoptosis of lung cancer cells via up-regulation of the double-stranded RNA-dependent protein kinase (PKR). *Cancer Res.*, **62**: 2239–2243, 2002.

111. Ito, T., Warnken, S. P., and May, W. S. Protein synthesis inhibition by flavonoids: roles of eukaryotic initiation factor 2 α kinases. *Biochem. Biophys. Res. Commun.*, **265**: 589–594, 1999.

112. Eberle, J., Fecker, L. F., Bittner, J. U., Orfanos, C. E., and Geilen, C. C. Decreased proliferation of human melanoma cell lines caused by antisense RNA against translation factor eIF-4A1. *Br. J. Cancer*, **86**: 1957–1962, 2002.

113. Polunovsky, V. A., Gingras, A. C., Sonenberg, N., Peterson, M., Tan, A., Rubins, J. B., Manivel, J. C., and Bitterman, P. B. Translational control of the antiapoptotic function of Ras. *J. Biol. Chem.*, **275**: 24776–24780, 2000.

114. D'Cunha, J., Kratzke, M. G., Alter, M. D., Polunovsky, V. A., Bitterman, P. B., and Kratzke, R. A. Over-expression of the translational repressor 4E-BP1 Inhibits NSCLC tumorigenicity *in vivo*. *Proc. Am. Assoc. Cancer Res.*, **43**: 816–817, 2002.

115. DeFatta, R. J., Nathan, C. A., and De Benedetti, A. Antisense RNA to eIF4E suppresses oncogenic properties of a head and neck squamous cell carcinoma cell line. *Laryngoscope*, **110**: 928–933, 2000.

116. Herbert, T. P., Fahraeus, R., Prescott, A., Lane, D. P., and Proud, C. G. Rapid induction of apoptosis mediated by peptides that bind initiation factor eIF4E. *Curr. Biol.*, **10**: 793–796, 2000.

117. DeFatta, R. J., Li, Y., and De Benedetti, A. Selective killing of cancer cells based on translational control of a suicide gene. *Cancer Gene Ther.*, **9**: 573–578, 2002.

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